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SOME EFFECTS OF POTASSIUM UPON THE GROWTH OF SUGAR CANE AND UPON THE ABSORPTION AND MIGRATION OF ASH CONSTITUENTS[1]

CONSTANCE ENDICOTT HARTT

(WITH ONE PLATE AND TWENTY-NINE FIGURES)

Introduction

An account was published in 1929 (**28**) dealing with the effect of varying amounts of potassium upon the growth, enzyme activity, moisture percentage, sugar content, cellular structure, and microchemistry of the sugar cane, variety Louisiana Purple. Suggestions were offered regarding the causes of the symptoms of potassium starvation, and an attempt was made to connect as cause and effect several derangements in the morphology and the physiology of sugar cane which were found to accompany the lack of potassium. The object of the present investigation was to repeat and expand the former study and thus to approach a better understanding of the rôle of potassium in the sugar cane plant.

The research program, of which this is the first report, included determinations of the enzymes invertase, amylase, and ereptase; analyses of moisture content, ash, total and amino nitrogen, reducing sugars and sucrose; the hydrogen ion concentration, titratable acidity, and titration curves of the juices expressed from the leaves, stems, and roots; some microchemical and histological observations, and studies conducted during the growth of the plants. As indicated by the title, this paper reports some effects of potassium upon the growth and ash constituents of sugar cane; the following paper deals with the nitrogenous and carbohydrate metabo-

[1] This investigation was conducted in 1931–1932 with the aid of the Sarah Berliner Research Fellowship of the American Association of University Women, at the Experiment Station of the Hawaiian Sugar Planters' Association, and is published at the expense of the Hawaiian Sugar Planters' Association. To both of these organizations the writer desires to express gratitude.

lism and enzyme activity of cane as affected by varying amounts of potassium.

METHODS.—*Saccharum officinarum*, variety H 109, was used in this investigation. This is the most important commercial variety grown in Hawaii at the present time under irrigated conditions. Healthy cane was gathered in the field July 14, 1931, cut to three-eye pieces and soaked in water for one hour. It was then given the hot water treatment (52° C. for 20 minutes) to destroy the stalk mite and to hasten germination. It was

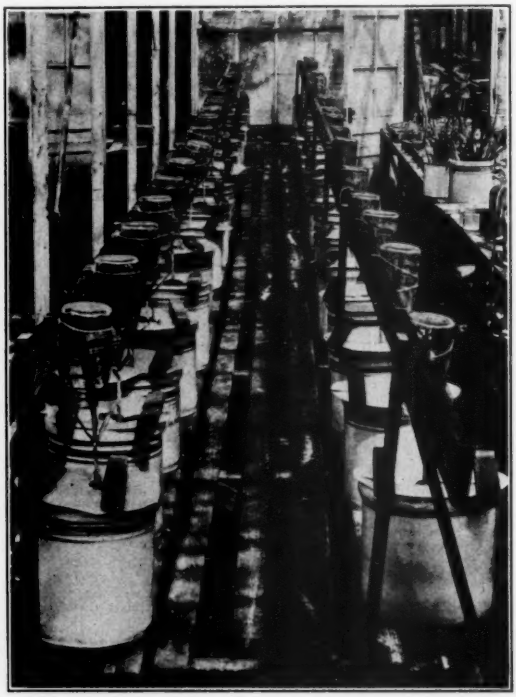


FIG. 1. Arrangement of sugar cane plants at time of transplanting, September 3, 1931.

planted in quartz sand in flats in the greenhouse and watered daily with tap water.

On September 3, 1931, young shoots were cut from the original "seed pieces," or cuttings, with about 1 inch of the cutting remaining attached to each plant, and were planted singly in 5-gallon, glazed, earthenware crocks and were watered with distilled water. The plants were not absolutely uniform at the time of transplanting, varying from 54 to 99 cm.

in length, this measurement being taken to the tip of the longest leaf. Most of the plants had well developed shoot roots. The few which were still dependent upon the seed-piece roots survived transplanting and grew as well as the others. The sand had been cleaned by the method described in the former paper. The pots were covered with overlapping pieces of lacquered cardboard, 4 mm. thick, with a central hole fitting the plant. This hole was enlarged and shaped when necessary to accommodate the developing secondary shoots. Drainage was maintained as described in the former paper. The condition and set-up of the experiment at the time of transplanting, September 3, 1931, are shown in figure 1.

Forty plants were grown in this experiment, eight in each of five different treatments. They were watered with the nutrient solutions for the first time on September 15, 1931. At first each plant received 1 liter of solution twice a week. This amount was increased from time to time, until in April, when the plants were finally harvested, the control plants were each receiving 3 liters every day. Because this amount was generally insufficient for the plants in series 2, there being none left to drain out the following day, these plants were each given 4 liters per day at the end of the experiment. The plants in the other series required less water because of their smaller size. The series of plants were numbered 1 to 5, corresponding to the solutions, in the order of decreasing amounts of potassium. Beginning January 13, 1932, two of the most poorly developed plants in series 4 and 5 were given the control solution regularly in order to study their recovery from potassium starvation. The two plants which were changed from solution 4 to solution 1 were then designated series 6; those changed from solution 5 to the control solution were then called series 7.

The plants were grown in a well ventilated glasshouse and were kept on ant-proofed benches.

The nutrient solutions were a modification of SHIVE's "best solution" (**65**) and were prepared from the following stock solutions:

KH_2PO_4	Volume molecular solution
$NaH_2PO_4 \cdot H_2O$	" " "
$Ca(NO_3)_2 \cdot 4H_2O$	" " "
$MgSO_4 \cdot 7H_2O$	" " "
$CaH_4(PO_4)_2 \cdot H_2O$	0.05 Volume molecular solution
$Ca(OH)_2$	Saturated solution

The composition of the nutrient solutions is given in table I.

The reaction of the solution varied from pH 6.3 to 6.5, using chlorphenol red and bromthymol blue as indicators.

Iron was originally added as ferric phosphate but by September 24 some of the plants were beginning to become chlorotic. Washing the

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS, IN CC. PER 2 LITERS

No. of solution	Potassium	Potassium phosphate	Sodium phosphate	Calcium phosphate	Calcium nitrate	Magnesium sulphate	Calcium hydroxide
	p.p.m.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1 (control)	87.9	4.5	.....	.....	10.4	10	50
2	39.0	2.0	2.5	.....	10.4	10	50
3	3.9	0.2	4.3	.....	10.4	10	50
4	0.0	.....	4.5	.....	10.4	10	50
5	0.0	.....	.....	44.8	10.4	10	50

chlorotic leaves with a 5 per cent. solution of ferrous sulphate produced a deeper shade of green within two or three days. Possibly ferric phosphate is not a satisfactory source of iron because it is too insoluble to supply the need of cane. Beginning October 7, 1931, enough iron in the form of ferrous sulphate was added to the sand to keep the plants green.

Since in the latter part of December the plants developed typical symptoms of Pahala blight (described later in this paper), the nutrient solutions were modified in several ways. Beginning January 4, 1932, 0.1 p.p.m. manganese was added to the solutions as $MnSO_4 \cdot 4H_2O$. Beginning March 3, the amount of manganese was doubled in solutions 1 and 2, and on March 23 the quantity was again doubled so that thereafter the plants in series 1 and 2 received 0.4 p.p.m. In addition, some of the leaves were washed with manganous sulphate, others with ferrous sulphate, and finally all of the leaves were dusted with a mixture of manganous sulphate and sulphur. The relative value of these treatments will be discussed later; suffice it to say at this point that by the time of the final harvest, April 27, the symptoms of Pahala blight had practically disappeared.

Because many of the sheaths showed a tendency to rot, this condition becoming apparent early in March, 1932, and because the ash analyses of the plants harvested on November 20, 1931, indicated that the leaves might be deficient in silicon, on March 22 dialyzed silica was added to the solutions. This was added at the rate of approximately 33 p.p.m. Si.

Several staff members of this Station have found that titanium aids in overcoming certain types of chlorosis in sugar cane (unpublished data). Beginning April 4, 1932, titanium nitrate was included in the solutions, at the rate of 10 p.p.m. Ti.

Several sugar cane insects gave trouble during the growing of the plants. These included the armyworm, *Spodoptera mauritia* Boisd.; the cane aphis, *Aphis sacchari* Zehnt.; the gray sugar cane mealybug, *Trionymus boninsis* Kuwana; the pink sugar cane mealybug, *Trionymus sacchari* Ckll.; the stalk mite, *Tarsonemus spinipes* Hirst; and the so-called red spider or leaf mite, *Tetranychus exsiccator* Zehnt. An attempt was made to combat the leaf mite and cane aphis by spraying the plants with distilled water but without success. The plants were thereafter sprayed once a week with Black Leaf 40. This was found to check the insects but not to prevent their development entirely.

Collection and preparation.—The plant material was harvested at three different intervals, being collected between 10 and 12 o'clock on sunny mornings.

On November 20, 1931, two plants of each of the series were harvested. The tops were cut from the roots, photographed, weighed, and separated into blades, sheaths, and stems. These were chopped separately in an

Athol chopping machine. Duplicate samples of blades and stems to be used for moisture determinations were placed in weighed aluminum boxes, treated with 1 cc. 95 per cent. alcohol, and weighed immediately. Weighed samples of blades and stems for sugar and nitrogen determinations were taken in duplicate, placed in Erlenmeyer flasks, each containing 1 gm. of calcium carbonate (to neutralize acids), and boiled with 100 cc. 95 per cent. alcohol. These samples were stored in the dark until ready for analysis. The remainder of the ground material was spread out between layers of filter paper and dried in a current of air at room temperature. It was then stored in desiccators in the dark. This material was used for enzyme determinations and for ash analyses. The roots were removed from the sand the following day and were dried without being ground.

On December 4, two plants from each of series 1 and 3 were removed from the crocks, the roots included. Photographs and weights were taken. The plants were separated into blades, sheaths, stems, and roots and were ground as just described, the sheaths being discarded. The ground material was placed in large Pyrex test tubes, stoppered, and the tops covered securely with wax paper. They were then frozen in a salt-ice mixture. The juice was expressed and used for determining the titratable acidity, hydrogen ion concentration, and titration curves, the results of which are reported in the second paper of this series.

On April 27, 1932, the final harvest was conducted, which consisted of all the remaining plants. The method was essentially the same as that of the November collection, except that the tops and roots were removed together from the sand. The blades were ground with a Russwin food cutter no. 3. The stems were sliced with a Sterling slicer no. 2 before being ground. As before, the roots were dried without being ground. Samples were taken in the same way as in the November harvest. The material for enzyme and ash analyses was dried in a current of air at a temperature not exceeding 43° C. and generally somewhat lower.

Results

1. Studies during growth

A. Symptoms of potassium deficiency.—The symptoms of potassium starvation which developed in this investigation were depressed growth of the entire plant, discoloration of the leaves, and dieback of the leaf tips. The plants grew rapidly and at first showed no real differences in their growth rates. Watering with the nutrient solutions was started on September 15, 1931. By October 7, *i.e.*, three weeks after starting the plants in the nutrient solutions, a slight gradation in growth was found, corresponding to the amount of potassium supplied, as shown in table II. This

gradation was noticeable in the figures for average size but could not be detected by just glancing at the plants. Growth in length is a measurement taken to the tip of the longest leaf. The total growth in length was determined by adding the growth in length of the main shoot to the total length of the tillers. This gave the total growth in length for the period from September 23 to October 7, because on September 23 the few secondary shoots were all still under the covers. Length measurements were all taken from the top of the cover because of the variation in the depth of the sand.

Differences in colors were hard to detect on October 7. Some of the lower leaves of the plants in series 4 and 5 were a little yellow, but whether this was the first discoloration due to potassium deficiency is hard to say because some of the leaves of series 1 were also slightly yellow.

On October 10 it was noticed for the first time that several of the leaves of the plants of series 3, 4, and 5 had conspicuous red areas on the upper surfaces of the midribs. There was no such discoloration on the midribs of series 1 and 2. The course of the development of red midribs and the histological studies and other investigations dealing with them will be considered later. They are illustrated in the plate.

The yellow condition of the lower leaves of the potassium-deficient plants became more pronounced. Gradually the edges of the leaves turned brown and died. This was accompanied by the dying and curling of the

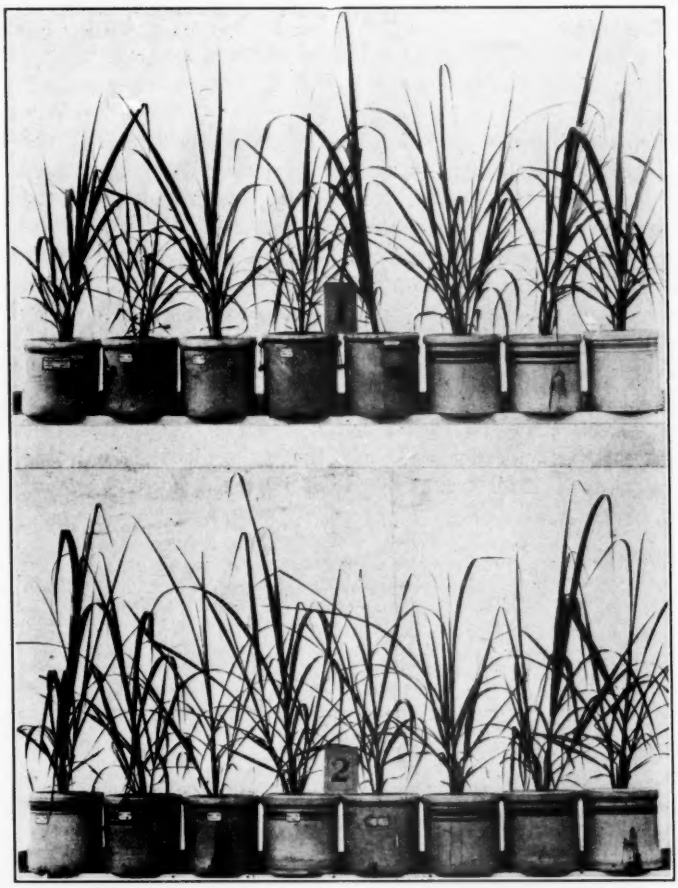
TABLE II

MEASUREMENTS OF PLANTS TAKEN OCTOBER 7, 1931, THREE WEEKS AFTER WATERING WITH NUTRIENT SOLUTIONS WAS BEGUN

SERIES	AVERAGE GROWTH IN LENGTH OF MAIN SHOOTS, SEPT. 23 TO OCT. 7	AVERAGE TOTAL LENGTH OF TILLERS, OCT. 7	AVERAGE TOTAL GROWTH IN LENGTH	AVERAGE NUMBER OF TILLERS
	cm.	*cm.*	*cm.*	
1	32.06	61.1	93.16	2.37
2	37.02	63.9	100.92	2.25
3	28.6	53.18	81.78	2.5
4	25.4	44.7	70.1	2.25
5	25.4	38.0	63.4	2.0

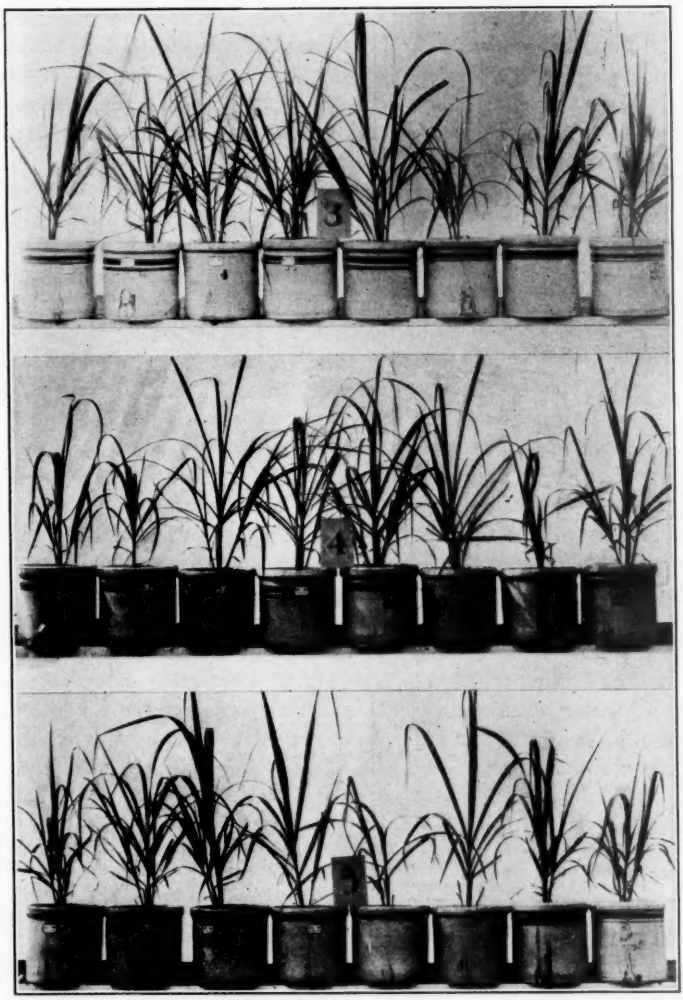
tips of the leaves, a condition known as dieback. Small red and red-brown spots developed in the laminae of the blades of the plants deficient in potassium. These symptoms were most fully developed in the plants of series 4 and 5, between which no difference could be observed. At first they were

not so conspicuous in series 3 as in 4 and 5; they never developed in series 1, and occurred only slightly in series 2 toward the end of the experiment. By October 23, the plants of series 3, 4, and 5 showed definite symptoms, including decreased growth, discoloration, and dieback. Figures 2 to



FIGS. 2 and 3. Series 1 (controls), on October 27, 1931, six weeks after starting them in the nutrient solutions. Series 2 on October 27, 1931.

6 show the plants on October 27, six weeks after starting them in the nutrient solutions. A definite gradation in size will be noted, as will also the similarity of the plants of series 4 and 5.



FIGS. 4–6. Series 3 (above) on October 27, 1931. Series 4 (middle) on October 27, 1931. Series 5 (below) on October 27, 1931.

The plate shows the typical colors of the plants. The sketches were made by a Station artist November 5 and 6, 1931, in the greenhouse from the living leaves. *A* shows a typical leaf from a control plant. It will be noted that there are some very small light green flecks on the lamina. Whether these are due to insect injury or are a similar condition (only less devel-

oped) to the red spots on the plants deficient in potassium was not ascertained. Histological studies by WELLER of this Station showed no mycelium. *B*, *C*, and *D* show the typical colors of the plants deficient in potassium; light, yellow-green laminae, yellow-brown edges, red midribs, red flecks in the laminae, and dieback. *B* shows the color of a midrib soon after it has become red, while *C* shows the darkening of the discoloration as the leaf ages. More will be said later regarding which of these may be considered primary and which secondary symptoms of potassium deficiency.

Symptoms of potassium deficiency under field conditions are the same as those already described. Similar symptoms also developed in the former investigation (**28**), with the exception of the red midribs. The yellow discoloration between the veins of the upper leaves occurring in the Chicago studies was probably due to a slight development of Pahala blight rather than to potassium deficiency. It would seem now that the symptoms of potassium deficiency in sugar cane are well established, comprising decreased growth of the entire plant, dieback of the leaf tips, and the discolorations of the blades as shown in the color plate. The brown edges and tips of the blades and yellowish green color of the laminae are primary symptoms while, as will be shown later, the red discoloration of the midribs is considered secondary.

The first harvest, consisting of two plants from each series, was conducted November 20, *i.e.*, about nine weeks after watering with the nutrient solutions was started. Averages of the measurements of the plants taken on November 19 are presented in table III. "Dewlap" is a term commonly applied in sugar cane parlance to the joint between the blade and the sheath. The height of the highest emerged dewlap is considered the best measurement of the growth of cane. According to VAN DEVENTER (**69**),

TABLE III

MEASUREMENTS TAKEN NOVEMBER 19, NINE WEEKS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS, IN AVERAGES

SERIES	LENGTH OF MAIN SHOOTS	TOTAL LENGTH OF TILLERS	HIGHEST DEWLAP	BREADTH OF LONGEST LEAF	NO. OF TILLERS	NO. OF RED MIDRIBS
	cm.	*cm.*	*cm.*	*cm.*		
1	189.4	566.7	38.6	4.1	12.0	0.0
2	192.6	787.7	36.7	3.9	13.0	6.7
3	163.9	332.9	25.5	3.1	2.7	17.0
4	140.2	181.0	20.1	2.7	2.2	10.5
5	145.1	243.7	20.3	2.7	2.5	19.7

the highest leaf joint is about 30 cm. above the real vegetative point because the sheath of that leaf is fully grown, or nearly so. Breadth of leaf is a measurement taken at the broadest part of the longest leaf; this is generally about the middle of the leaf. The average growth of the plants for the period November 3 to 19 is given in table IV. Fresh weights of blades, sheaths, and stems, and air-dry weights of roots of the plants collected November 20 are given in table V. Tables III to V show that nine weeks after starting the plants in the nutrient solutions, the plants of series 1 and 2 were considerably larger in every measurement than the plants starved for potassium, series 3 to 5.

TABLE IV

AVERAGE GROWTH, NOVEMBER 3 TO 19

SERIES	LENGTH OF MAIN SHOOTS	HIGHEST DEWLAP	LENGTH OF TILLERS	NO. OF TILLERS
	cm.	*cm.*	*cm.*	
1	15.4	7.7	262.6	5.5
2	17.5	5.0	464.2	5.0
3	14.9	2.1	73.8	Decreased in number
4	4.9	0.0	0.0	"
5	12.7	0.2	55.7	"

TABLE V

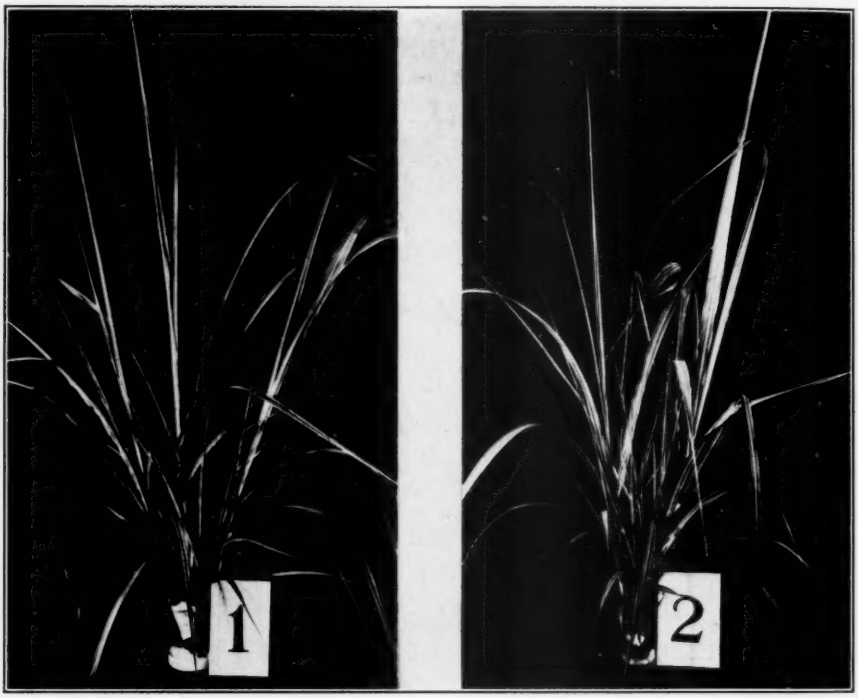
AVERAGE WEIGHTS OF PLANTS, NOVEMBER 20

SERIES	FRESH WEIGHT				AIR-DRY WEIGHT
	BLADES	SHEATHS	STEMS	TOTAL TOPS	ROOTS
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
1	256.0	188.0	81.0	526.0	23.7
2	269.2	196.0	86.2	551.0	28.7
3	130.0	71.2	24.0	225.2	11.2
4	68.0	30.0	9.2	107.7	5.8
5	78.7	35.0	14.0	128.2	6.0

In most measurements the plants of series 2 averaged larger than those of series 1. Attention is drawn to the close similarity between the plants of series 4 and 5; where there is a difference between them it is in favor of series 5. Evidently sodium does not replace potassium in the growth of

sugar cane. Figures 7 to 11 show the plants at the time of harvesting, November 20, 1931.

Besides the colors, which are shown in the plate, several other differences should be mentioned. There was a gradation in the development of wax on the stems correlated with the supply of potassium. The roots showed a



FIGS. 7 and 8. Typical plant (left) of series 1 (control) on November 20, 1931, nine weeks after starting the plants in the nutrient solutions. Typical plant (right) of series 2 on November 20, 1931.

gradation in size and condition, those of series 1 and 2 being large, firm, and white and those of the plants deficient in potash being small, poorly developed, and discolored yellow and brown.

The second harvest was conducted December 4, eleven weeks after starting the plants in the nutrient solutions. At this time two plants from each of series 1 and 3 were collected. Table VI gives the average measurements of all the plants taken December 3. Table VII gives the weights of the tops and roots of the plants harvested December 4, 1931.

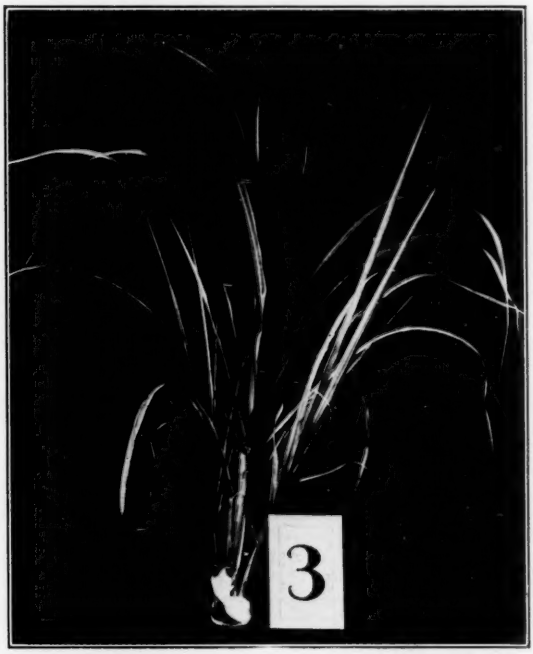


FIG. 9. Typical plant of series 3 on November 20, 1931.

Figures 12 and 13 illustrate the plants just after they were removed from the crocks.

On January 13, about four months after starting the plants in the nutrient solutions, two of the most poorly developed plants of series 4 and 5 were

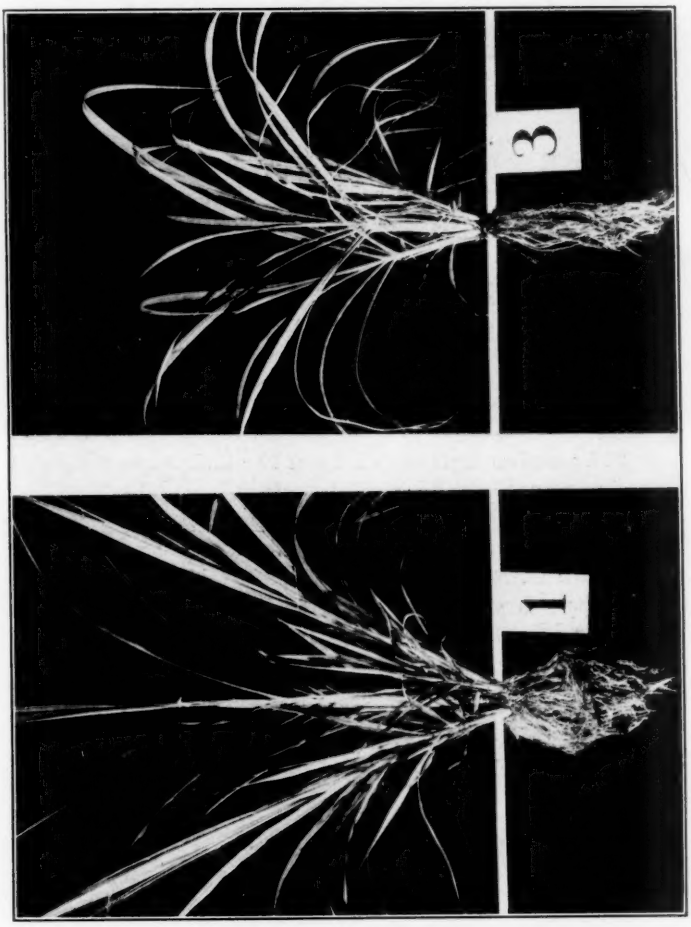
TABLE VI

AVERAGE SIZE OF PLANTS, DECEMBER 3, ELEVEN WEEKS AFTER STARTING SOLUTIONS

SERIES	LENGTH OF MAIN SHOOTS	BREADTH OF LONGEST LEAF	HIGHEST DEWLAP	NO. OF TILLERS
	cm.	*cm.*	*cm.*	
1	196.1	4.5	47.2	11.5
2	198.9	4.2	42.5	12.5
3	165.0	3.0	25.8	4.5
4	143.5	2.5	21.3	3.0
5	150.1	2.7	22.3	2.5

FIGS. 10 and 11. Typical plant (above) of series 4 on November 20, 1931. Typical plant (below) of series 5 on November 20, 1931.

given solution 1, this treatment being continued to the end of the experiment. Their appearance on January 13, before starting the new treatment, is shown in figure 14. Thereafter these were called series 6 and 7 respectively. They immediately started to grow. The stems which were already formed became more rigid, straightened up, lengthened, and increased in circumference. Adventitious roots developed at the base of the stem, which acted like prop roots in supporting the rapidly developing stems. Buds also developed at the bases of the stems, and quickly formed new shoots.



FIGS. 12 and 13. Typical plant (left) of series 1 on December 4, 1931. Typical plant (right) of series 3 on December 4, 1931.

The old leaves, which exhibited the symptoms of potash deficiency, dried up, died and fell down; the newly developed leaves were as green and nearly as large as those of the plants of series 1. The increased growth of the stems is shown graphically in figure 15. By the latter part of February, these plants had overtaken and surpassed the plants of series 3 in development. It is interesting that series 7 showed a greater capacity for recovery than did series 6. Their measurements at the time of the final

TABLE VII

WEIGHTS OF TOPS AND ROOTS, DECEMBER 4

PLANT	TOPS	ROOTS
	gm.	*gm.*
1 G	800.0	448.0
1 H	885.0	354.9
3 G	222.0	201.0
3 H	265.5	246.0

harvest, April 27, are given in tables VIII and IX; their appearance at that time is shown in figures 21, 22, 28, and 29.

The final harvest of all the plants was conducted April 27, seven and one-half months after starting the plants in the nutrient solutions. Size measurements taken on April 25 are presented in table VIII. The fresh weights of the tops collected April 27 and the air-dry weights of the roots are given in table IX. Figures 16–22 show all the plants on April 25.

FIG. 14. Appearance of series 4 and 5 on January 13, 1932, just before changing to solution 1. Thereafter these plants were called series 6 and 7 respectively.

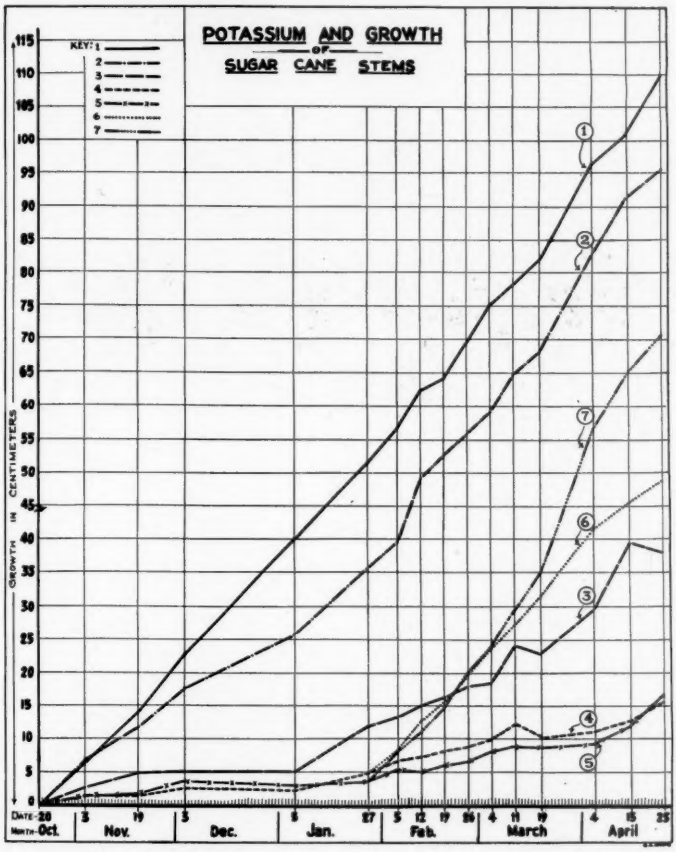
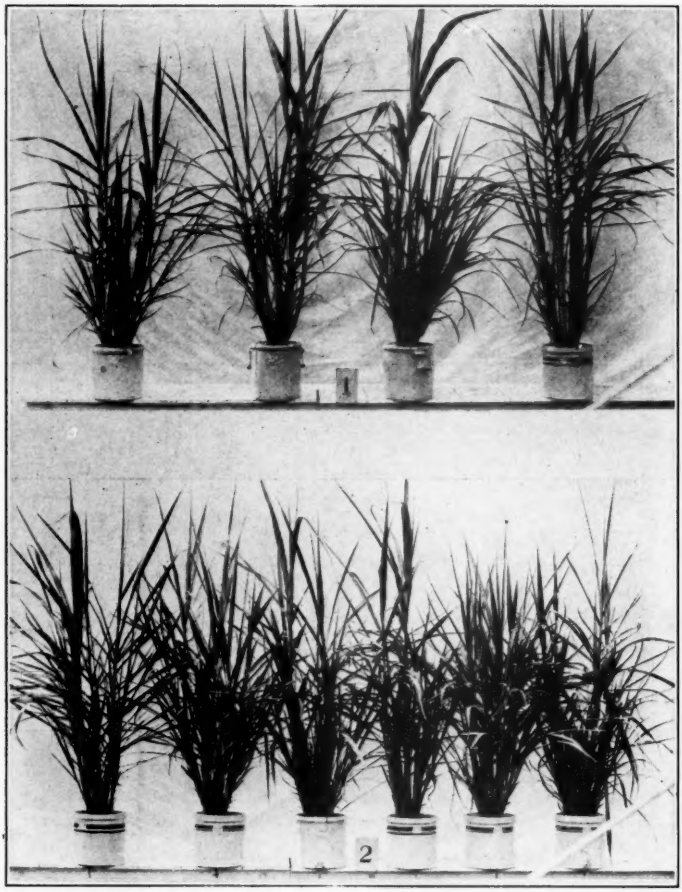


FIG. 15. Effect of potassium upon growth of stems as indicated by the average height of the highest emerged "dewlap" (juncture of blade and sheath).

Photographs of representative plants taken at the final harvest are shown in figures 23–29. The colors of the plants were practically the same as before, except that some of the leaves of the plants of series 1 and 2 were burned, owing to the high temperature in the greenhouse. The plants showed very little Pahala blight, these symptoms being confined chiefly to the old leaves of the secondary shoots. Most of the plants deficient in potassium had red midribs. The plants of series 6 and 7 had the best developed individual roots, because they were not yet potbound. Some of the longer roots showed plainly where they had begun to increase growth

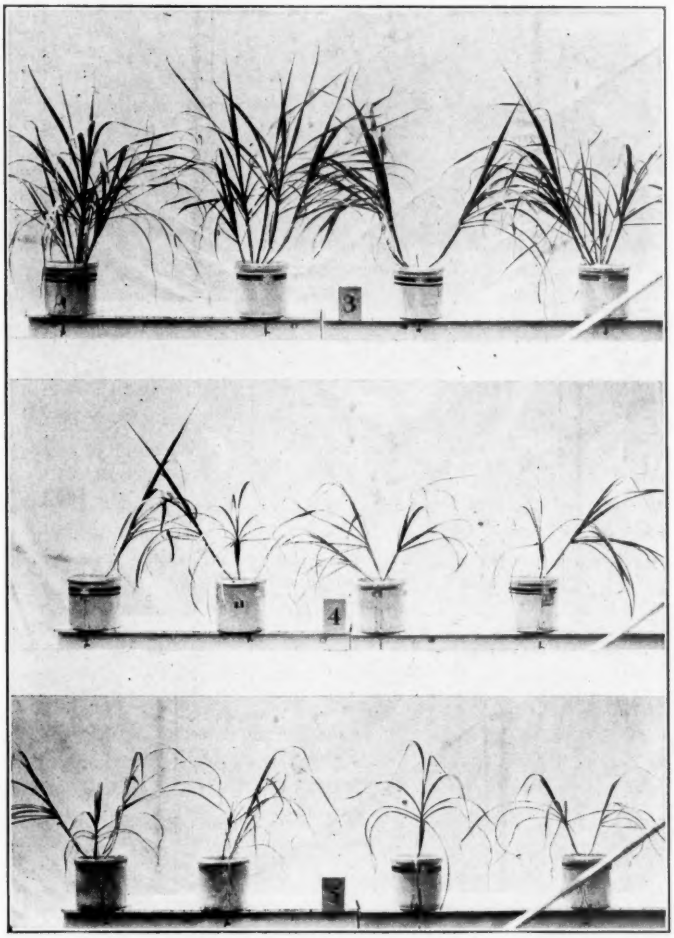


FIGS. 16 and 17. Series 1 (above) on April 25, 1932, 7½ months after starting them in the nutrient solutions. Series 2 (below) on April 25, 1932.

at the time of the first addition of potassium. The plants of series 1 and 2 had very large and interwoven masses of roots.

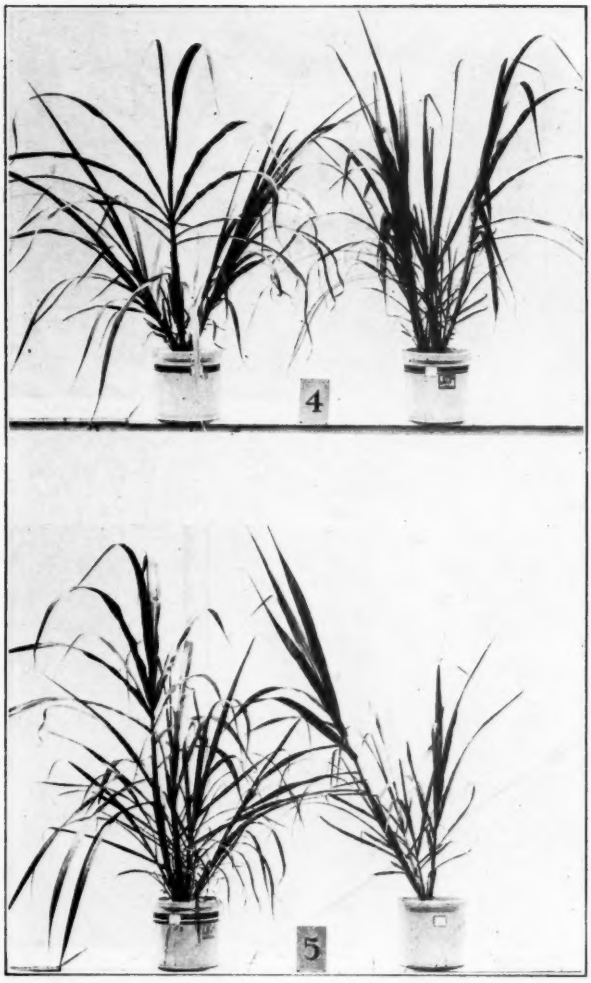
Graphs of the increase in height of the highest emerged dewlap are given in figure 15. It will be noted that the growth of the stems is markedly affected by the amount of potassium supplied. After the first week or so the plants of series 1 maintained a steady growth, always greater than that of any of the others. The speedy recovery of the plants of series 6 and 7 after the addition of potassium in January is conspicuous. The

increase in growth of series 3, 4, and 5 beginning in January may be due in part to the increased light in the spring, but is probably due also to the fact that manganese was added to the solutions for the first time on January 4.



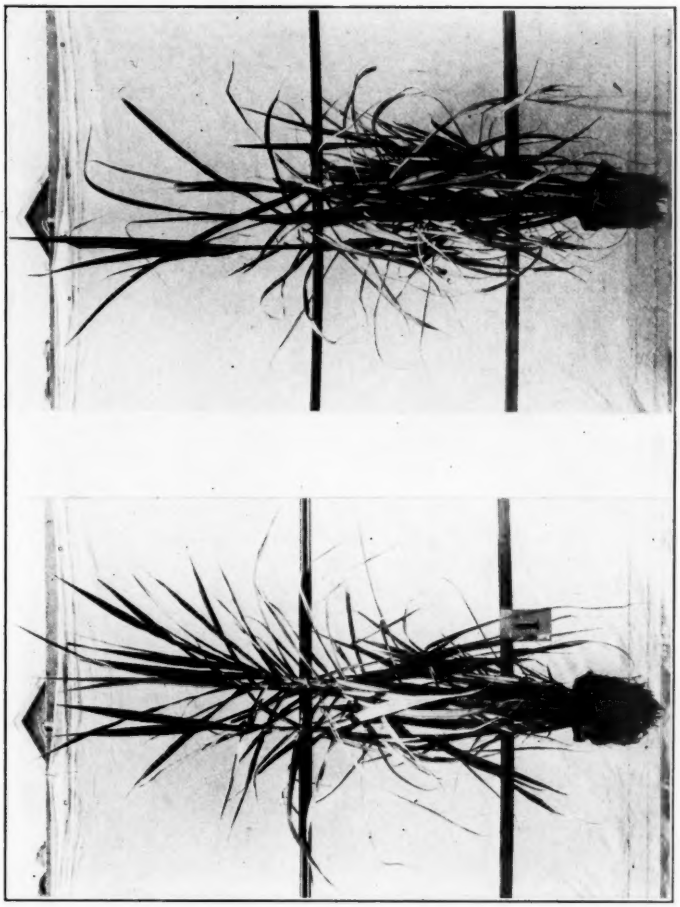
FIGS. 18–20. Series 3 (above) on April 25, 1932. Series 4 (middle) on April 25, 1932. Series 5 (below) on April 25, 1932.

That manganese is essential for the growth of sugar cane has been shown by DAVIS (**15**) and MARTIN (unpublished work). The development of Pahala blight in the absence of manganese has been studied by LEE and MCHARGUE (**45**) and by MARTIN (**48**).

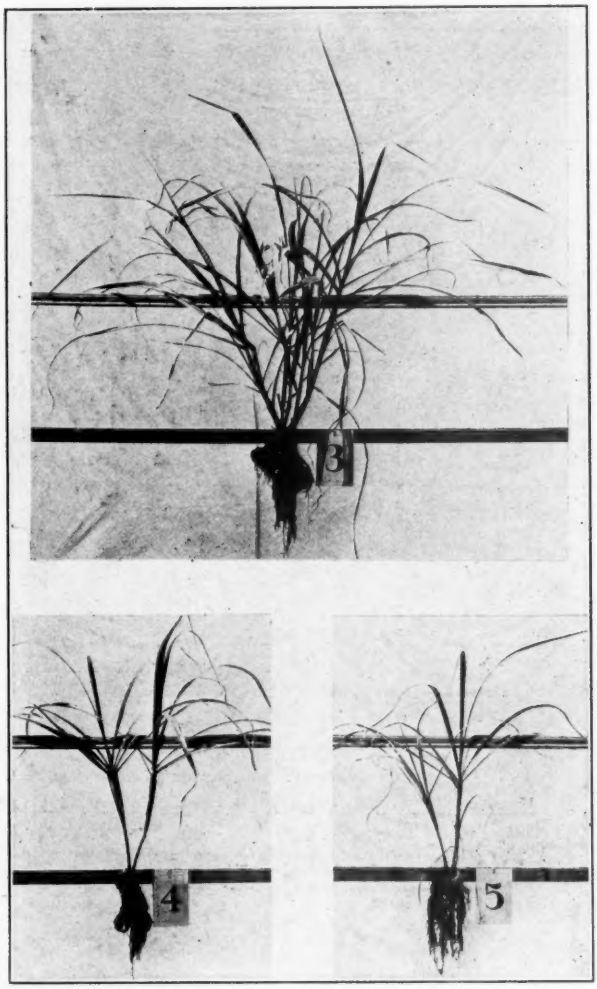


FIGS. 21 and 22. Series 6 (above) on April 25, 1932. Until January 13, these plants received solution 4; after that time, solution 1. Series 7 (below) on April 25, 1932. Until January 13, these plants received solution 5; after that time, solution 1.

Mention has already been made of the development of red midribs on the leaves of plants deficient in potassium. The average numbers of red midribs per plant are shown in table X. These counts were discontinued after November 22. No red midribs developed in the plants of series 1. In

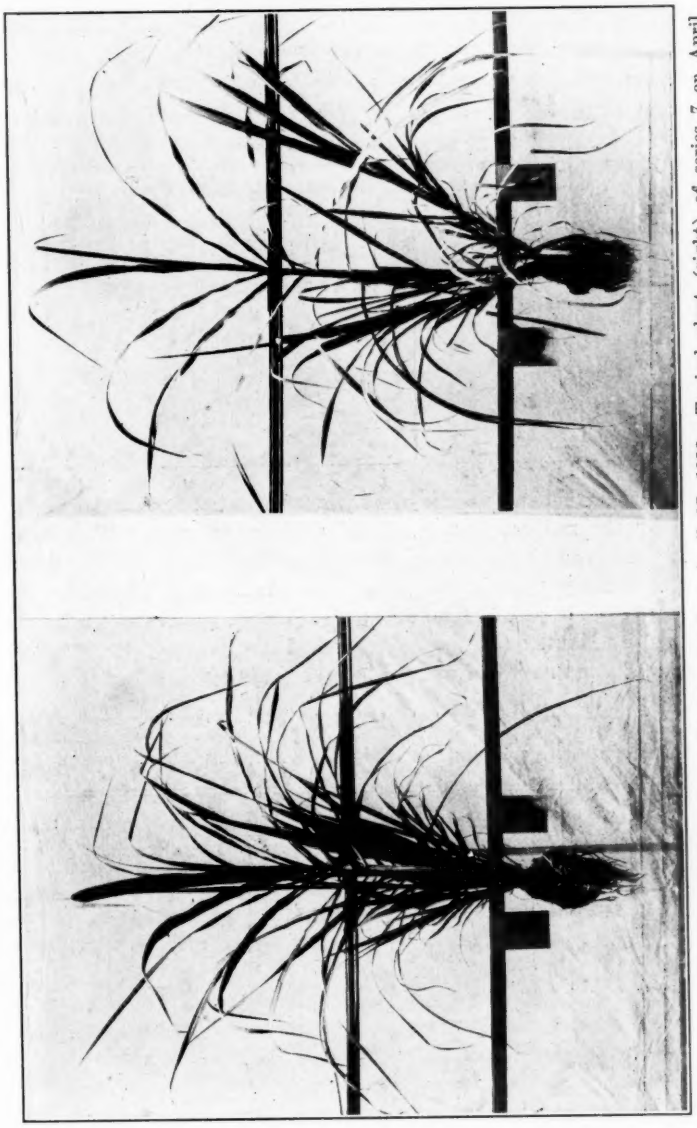


Figs. 23 and 24. Typical plant (left) of series 1 at the final harvest, April 27, 1932. Typical plant (right) of series 2 on April 27, 1932.



FIGS. 25–27. Typical plant (above) of series 3 on April 27, 1932. Typical plant (below, left) of series 4 on April 27, 1932. Typical plant (below, right) of series 5 on April 27, 1932.

general, they occurred on most of the plants of series 3, 4, and 5 up to the end of the experiment. It will be seen that their occurrence is correlated with a deficiency in potash. The question arose as to whether this discoloration is a specific symptom of potash deficiency in cane or a secondary reaction.



FIGS. 28 and 29. Typical plant (left) of series 6 on April 27, 1932. Typical plant (right) of series 7 on April 27, 1932.

TABLE VIII

AVERAGE SIZE OF PLANTS AT FINAL HARVEST, APRIL 25, 7½ MONTHS AFTER STARTING IN THE NUTRIENT SOLUTIONS

SERIES	HIGHEST DEWLAP OF MAIN STALKS	BREADTH OF LEAF AT MIDDLE OF LONGEST LEAF OF MAIN STALK	CIRCUMFERENCE OF MAIN STALKS
	cm.	*cm.*	*cm.*
1	130.9	5.5	9.3
2	116.4	4.4	8.4
3	57.9	3.9	5.5
4	34.3	2.6	3.8
5	36.0	2.6	3.7
6	69.0	4.9	7.7
7	94.3	5.6	9.3

Red midribs frequently occur on cane grown in the field resulting from infection by the red rot fungus, *Colletotrichum falcatum*. This fungus gains entrance through mechanical injuries or following leafhopper injury. The plants of the present investigation were examined by the pathologists and entomologists of this Station and no leafhopper punctures were found. As reported by MARTIN (**47**), samples studied histologically by WELLER of this Station showed no mycelium. Attempts made by MARTIN to isolate an

TABLE IX

AVERAGE WEIGHTS OF PLANTS COLLECTED APRIL 27, EXPRESSED IN GRAMS

SERIES	FRESH WEIGHT				AIR-DRY ROOTS
	BLADES	STEMS	SHEATHS	TOTAL TOPS	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
1	955.88	2185.10	1009.75	4150.73	108.5
2	1035.30	1639.50	993.60	3668.40	106.8
3	277.70	176.95	172.92	627.57	27.8
4	62.02	25.20	28.30	115.52	8.2
5	63.70	28.05	31.87	123.62	11.3
6	569.75	358.25	443.00	1371.00	47.6
7	472.50	415.25	337.00	1224.75	40.5

TABLE X

AVERAGE NUMBER OF RED MIDRIBS

	SERIES				
	1	2	3	4	5
October 15	0	0	1.5	2.3	1.5
October 20	0	0	4.8	4.7	5.6
November 4	0	0.6	12.7	12.6	11.8
November 22	0	6.7	17.0	10.5	19.7

organism from red-midrib tissue were negative. Hypodermic injections of 0.25 per cent. acetic acid into the midribs of other cane plants did not result in the development of any red discoloration. It was concluded that the red midribs were not caused by an organism or by acidity alone.

Microscopic studies showed that the red color of the midribs was a discoloration of the lignified cell walls. The cuticle and epidermis were not affected. The walls of the subepidermal plates were red, also the sheaths of the large vascular bundles, the xylem, and the lignified regions between the bundles and the lower epidermis. The color of the walls was the same as that which develops in the microchemical test for lignin, *viz.*, staining with phloroglucin-hydrochloric acid. The course of the discoloration seemed to be as follows: first the protoplasm of the parenchyma became slightly brown, then the parenchyma walls next to the lignin became faint red, finally the lignified walls reddened. Thus the disturbance seemed to originate in the parenchyma.

STEUERWALD (**67**) isolated saccharetin from cane fiber and stated that it belongs to the phlobaphenes and may be considered the aromatic compound in lignin. It is very soluble in alcohol. On treatment with phloroglucin-hydrochloric acid, saccharetin becomes intensely red.

Because of these characteristics of saccharetin and because of the resemblance of the discolored walls to the ordinary lignin test, it was thought that phloroglucin might be present in the cells of the potassium-deficient leaves, and thus explain their discoloration. Phloroglucin occurs in some plants as a hydrolytic product of certain tannic acids (**23**).

Evidence of the presence of phloroglucin was obtained as follows. Heating sections of the discolored midribs in alcohol partially removed the red color. When a partially decolorized section was treated with concentrated hydrochloric acid, with no addition of phloroglucin, the reddish color deepened. Subsequently heating caused a decided reddening of the walls. When sections of the control leaves were heated with hydrochloric acid no

suggestion of red occurred, although decisive tests for lignin were obtained using phloroglucin-hydrochloric acid. It would seem that the plants of series 1 contained no phloroglucin, while the potassium-deficient plants did.

The need for an acid as well as phloroglucin for the red coloration of saccharetin was realized. The presence of enough hydrochloric acid within the plants was doubted, since the nutrient solutions contained no chlorides. The attempt was made to produce a similar color using tannic acid instead of hydrochloric, but without success. Sulphuric acid, however, gave a red color very similar to that formed when hydrochloric acid was used, and closely resembling the discolored midribs, both in shade and location. The first red midribs to develop were noticed on October 10, just three days after the first application of ferrous sulphate in the nutrient solutions. It would appear that this offers an explanation of the red midribs of the potassium-deficient plants.

To sum up, it seems possible that potassium deficiency causes a decomposition of tannic acids, resulting in the formation of phloroglucin. This in the presence of an excess of sulphuric acid may cause the reddening of saccharetin. The development of red midribs is thus a secondary rather than a primary symptom of potassium deficiency, and will occur only in the presence of an excess of sulphates or possibly chlorides.

Since the above was written, confirmatory evidence of the importance of sulphates in the development of red midribs has been supplied by W. W. G. Moir, of American Factors, Honolulu, who states that at Olaa Sugar Company red midribs developed within a month after the application of two tons of sulphur per acre to land deficient in potassium. The midribs of other plants became red after the application of copper sulphate.

In this connection it is interesting to note that it is characteristic of sugar cane to become reddened when diseased or injured, whatever the causal agent. Although other factors are probably involved also, the possibility is suggested that wherever a red color develops in the cell walls, following disease or injury, it may be due to the action of sulphates or chlorides and phloroglucin upon saccharetin. Sugar cane soils are often high in sulphates, and it seems possible that many diseases might lead to the decomposition of tannic acids and thus the formation of phloroglucin.

Van Den Honert (**70**) recently described symptoms of potassium deficiency obtained with variety P. O. J. 2878, which in general agree with those described here. The main difference between the Java results and ours lies in the location of the discoloration of the midribs. Van Den Honert gives figures of cross sections of the discolored midribs and states that in potassium deficiency the discoloration is confined to the upper surface; whereas all the lignified regions of the midribs of the potassium-deficient plants appeared red in places in the investigation herein reported,

when examined microscopically. It is interesting to note that VAN DEN HONERT used sulphuric acid in his nutrient solutions, because of the importance of sulphates in the production of red midribs brought out in this study. The gumming of the epidermal walls of the midribs considered a symptom of potassium starvation by VAN DEN HONERT was not observed in the present study.

B. ABSORPTION OF POTASSIUM.—Three experiments were conducted dealing with the absorption of potassium. On October 16, composite samples of the drainage of the plants in series 1 to 3 were collected. Each plant had received 1.5 liters of solution four days previously. Analyses for potassium were performed by the Chemistry Department of this Station and the results are given in table XI. The control solution prepared that week contained 92 p.p.m. potassium, by analysis. Analyses of the other solutions were not made, the calculated parts-per-million being used to obtain the amount of potassium absorbed by the plants of series 2 and 3. It will be noted that the plants of series 1 and 2 absorbed potassium at the same rate that week.

TABLE XI

POTASSIUM ANALYSES OF DRAINAGE COLLECTED OCTOBER 16, 1931

SERIES	K IN DRAINAGE	K ABSORBED IN 4 DAYS
	p.p.m.	*p.p.m.*
1	62.0	30.0
2	9.4	30.0
3	2.1	1.8

Several investigators have found evidence of a relationship between the absorption and utilization of potassium and light, which will be mentioned more completely in the discussion. To determine whether the amount of potassium absorbed during the day differs from that taken up by night, the following test was performed. On November 13 and 14, drainage samples were collected from the plants of series 1 and 2 at 6 A. M. and 6 P. M. Fresh solutions were added each time. The samples were analyzed for potassium by the Chemistry Department of this Station. Sunrise occurred at 6:11 and sunset at 5:20, according to the Weather Bureau. It was therefore possible to determine the amount of potassium absorbed during approximately 12 hours of darkness and 12 hours of daylight. The results are reported in table XII, which shows that both sets of plants absorbed the same amount of potassium during the night and the day.

TABLE XII

DAY AND NIGHT ABSORPTION OF POTASSIUM

SERIES	WATERED 6 P. M. FRIDAY AND 6 A. M. SATURDAY	CONTAINED 6 A. M. SATURDAY	CONTAINED 6 P. M. SATURDAY	ABSORBED IN 12 HOURS' DARKNESS	ABSORBED IN 12 HOURS' DAYLIGHT
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	93.6	4.7	5.2	89.9	88.4
2	43.1	3.0	3.5	40.1	39.6

The object of the third experiment dealing with absorption was to determine whether or not cane plants can absorb potassium through their leaves. It is well known that iron can be absorbed that way. Evidence has been presented by SHAW (**63**) showing that cane leaves may absorb large quantities of water during rains. DOMONTOVICH and ZHELEZNOV (**17**) have shown that painting leaves with salts of potassium and magnesium leads tc the absorption of these elements.

On March 18, several leaves of the plants of series 5 were washed with a 5 per cent. solution of potassium dihydrogen phosphate. No sign of recoloration was observed. The results of this test are inconclusive, since it is possible that potassium might be absorbed without causing the leaves to become green. On the other hand, it may be that they were already beyond recovery.

C. HYDROGEN ION CONCENTRATION OF DRAINAGE.—Colorimetric determinations of the hydrogen ion concentration of the drainage were made at intervals. The reaction of the nutrient solutions was pH 6.4 and the object of these tests was to ascertain how well this reaction was maintained. The results are presented in table XIII. The high acidity was probably due to

TABLE XIII

PH OF DRAINAGE

SERIES	COMPOSITE DRAINAGE					INDIVIDUAL	
	OCT. 23	OCT. 26	OCT. 30	NOV. 2	NOV. 6	NOV. 9	NOV. 11
1	3.4	3.3	3.9	3.9	4.4	6.5–7	5.9–7.1
2	3.6	3.7	4.6	6.1	5.6	7	6.9
3	3.6	4.2	4.3	4.1	4.7	3.9–6.1	.....
4	3.7	2.8	3.7	3.9	4.3	3.5–6.1	.....
5	3.5	2.8	3.5	3.7	4.0	3.6–5.9	.....

the use of ferrous sulphate as the source of iron. Because of the acidity, the solutions were changed more frequently. An important fact is that there was as much variation in reaction between the individuals of one series as there was between the different series, indicating that the differences in growth and external appearance of the plants were not caused by differences in the reaction of the medium. There was no correlation within a series between hydrogen ion concentration and general appearance, length of leaf, height of dewlap, number of red midribs, or amount of dieback. McGeorge (**51**) found that a hydrogen ion concentration as acid as pH 4.0 was without effect upon the growth of cane. An important conclusion to be drawn from these tests is that cane can grow in quartz sand cultures at a reaction varying from about pH 3.5 to 7 without any apparent external differences. In the field the range is probably less than that, since under field conditions changes in hydrogen ion concentration would cause more or less aluminum to go into solution, which would have a decided effect upon growth, as mentioned by Moir at the 52d annual meeting of the Hawaiian Sugar Planters' Association, December, 1932.

D. Pahala blight.—The symptoms of Pahala blight which began to develop the latter part of December, 1931, were alternating stripes of green and white in the blades, small elongated red spots in the white stripes, longitudinal splitting of the blades, and cessation of growth of the secondary stems. These symptoms are the same as those described by Lee and McHargue (**45**). They were confined to the plants of series 1 and 2.

The addition of 0.1 p.p.m. manganese as manganous sulphate to the nutrient solutions, begun January 4, resulted in a diminution of the symptoms of the blight. The secondary shoots started growing again. Because of a recurrence of the symptoms, the amount of manganese in solutions 1 and 2 was doubled twice in March.

Washing the leaves with 1 and 3 per cent. solutions of ferrous sulphate and manganous sulphate on March 4 and 19 resulted in no recoloration of the leaves. On March 21, plant 1 E was dusted with a mixture composed of equal quantities of manganous sulphate and sulphur. In two days this plant looked better. Thereafter all the plants of series 1 and 2 were dusted once a week. By the time of the final harvest the symptoms of Pahala blight had practically disappeared.

E. Silicon and titanium.—As mentioned earlier, silicon was added to the solutions beginning March 22, 1932. This was deemed necessary because of the rotting of the sheaths. On March 28 it was noted that the sheaths, particularly those of the potassium-deficient plants, were stronger, stiffer, greener, and less inclined to rot. Early in April it was noted that none of the plants had as many dead leaves as before the use of silicon. Although the addition of silicon was not a controlled experiment, the evi-

dence presented here seems to indicate that it is essential for the best growth of cane.

There was no evidence that the addition of titanium nitrate to the solutions produced any effect upon the growth or appearance of the plants.

2. Microchemical observations

The stems of the potassium-deficient plants were found to contain iron at the nodes when given the Hoffer test (**33**), while the stems of the controls showed very little accumulation of iron. A dark discoloration of the bundles of the stems of the plants deficient in potassium was noted before staining. The sieve tubes and companion cells of some of the bundles of the midribs of the plants of series 5 were found to be very dark brown, when examined October 29, 1931. These discolorations were not found in the control plants.

3. Analytical data

The results of the moisture determinations are found in table XIV. In general, the plants supplied with potassium had a higher percentage of water than the potassium-deficient plants.

TABLE XIV

Moisture percentages in cane

Series	November harvest		April harvest	
	Blades	Stems	Blades	Stems
	%	%	%	%
1	74.1 ± 0.071	83.4 ± 0.047	79.1 ± 0.333	86.35 ± 0.071
2	74.9	85.6 ± 0.023	74.65 ± 0.071	83.65 ± 0.548
3	72.1 ± 0.238	81.0 ± 0.381	75.3 ± 1.09	79.7 ± 0.095
4	.....	.....	76.85 ± 0.071	80.75 ± 0.071
5	.....	.....	76.85 ± 0.548	76.75 ± 0.023
6	.....	.....	78.15 ± 0.023	.....
7	.....	.....	77.65 ± 0.071	.....

The results of the ash analyses, which were performed by the Chemistry Department of this Station, are given in tables XV and XVI, in which they are expressed on the percentage basis. The percentage of potassium within the plants was directly proportional to the amount supplied in the nutrient solution. In November the percentage of total ash was higher in the plants deficient in potassium than in the controls, in both blades and roots, whereas in April the blades of the control plants had the highest percentage of ash, the amount in the roots remaining (as before) greater in the potassium-deficient plants. In November the blades of the plants deprived of potash

had the highest percentages of phosphorus, calcium, and magnesium; in the roots, phosphorus was greater in the potassium-deficient plants, but calcium and magnesium did not vary greatly. Silicon varied inversely with the amount of potassium in the blades, while in the roots the percentage of silicon varied irregularly, in the November material. While the high percentages of silicon in the roots in both the November and April material might suggest the possibility of contamination with minute particles of quartz sand, yet every precaution was taken to prevent such contamination and the chemist stated that no grains of sand were found in the ash.

In April the percentage of potassium in the blades, stems, dead leaves, and roots varied directly with the amount supplied. It is interesting that

TABLE XV

ASH ANALYSIS OF PLANTS HARVESTED NOVEMBER 20, 1931
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	ASH	SI	K	P	CA	MG
	%	%	%	%	%	%
Blades						
1	5.139	0.0659	1.78	0.359	0.279	0.170
2	4.222	0.0617	0.955	0.396	0.411	0.384
3	4.666	0.0892	0.540	0.668	0.502	0.540
4	5.636	0.121	0.424	0.882	0.503	0.645
5	5.917	0.100	0.458	0.983	0.557	0.521
Roots						
1	24.96	8.008	1.78	0.598	0.532	0.308
2	17.42	4.555	0.741	0.450	0.765	0.471
3	28.56	9.395	0.388	0.747	0.515	0.274
4	31.74	11.42	0.294	0.721	0.550	0.237
5	33.82	10.99	0.329	0.856	0.616	0.268

the plants of series 7 contained a higher percentage of potassium in the blades, stems, and roots than the controls. The percentage of potassium in series 6 also surpassed that in series 1 in stems and roots. Evidently the plants which received the first application of potassium in January absorbed it at a high rate.

In April phosphorus was again found to be higher in the potassium-deficient plants, in general. The percentage of silicon was inversely proportional to the potassium content in the roots, in the April material. Calcium, which was higher in the potassium-deficient plants in November, showed the opposite relationship in the blades in April but no striking differences in the other organs. Magnesium was higher in the blades and stems of the potassium-deficient plants than in those of the controls.

The plants receiving sodium contained more of that element than the other plants, but except in the stems the percentage of sodium in the plant was not proportional to the amount supplied in the nutrient solutions. In general the percentages of sodium decreased from the roots to the stems to the blades, whereas the percentages of potassium increased. All the blades and the stems contained higher percentages of potassium than sodium, even those supplied with more sodium than potassium. With the exception of the plants of series 3 and 5 the same condition existed in the roots.

There were greater percentages of iron in the blades, stems, and roots of the plants deficient in potassium than in the controls. The amount of

TABLE XVI

ASH ANALYSIS OF PLANTS HARVESTED APRIL 27, 1932
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	ASH	SI	K	P	CA	MG	NA	FE
	%	%	%	%	%	%	%	%
Blades								
1	7.34	0.118	2.175	0.5102	0.544	0.4301	0.014	0.002
2	7.88	0.094	1.801	0.5341	0.842	0.5621	0.037	0.017
3	5.61	0.103	0.724	0.6529	0.278	0.5163	0.030	0.054
4	6.46	0.130	0.355	0.8007	0.215	0.6379	0.033	0.031
5	6.41	0.174	0.501	0.7893	0.241	0.5412	0.026	0.052
6	6.47	0.151	1.765	0.5269	0.279	0.2717	0.025	0.031
7	6.87	0.164	2.475	0.4964	0.264	0.2590	0.025	0.064
Stems								
1	4.53	0.119	1.504	0.4242	0.116	0.1828	0.020	0.020
2	3.18	0.158	0.437	0.3593	0.191	0.2710	0.129	0.016
3	2.98	0.102	0.335	0.4227	0.149	0.2547	0.143	0.063
4	3.77	0.134	0.292	0.5262	0.167	0.2391	0.237	0.254
5	4.22	0.153	0.278	0.5572	0.168	0.2941	..... *	..... *
6	6.21	0.261	1.934	0.5215	0.131	0.4119	0.015	0.025
7	5.99	0.057	2.206	0.5050	0.106	0.3376	0.019	0.019
Dead leaves								
1	8.23	0.102	1.270	0.6335	0.632	0.6115	0.025	0.047
2	8.78	0.111	0.213	0.7048	1.395	0.4881	0.205	0.059
3	7.58	0.123	0.160	0.9481	0.179	0.6236	0.041	0.031
4	7.81	0.140	0.205	0.9565	0.287	0.7248	..... *	0.041
5	8.53	0.117	0.132	0.1429	0.112	0.5588	0.027	0.027
6	8.54	0.145	1.262	0.8530	0.527	0.6050	..... *	..... *
7	8.61	0.175	1.154	0.8725	0.426	0.5630	..... *	..... *
Roots								
1	12.14	1.904	0.901	0.7817	0.991	0.2135	0.082	0.233
2	14.88	2.975	0.353	0.8123	1.254	0.2851	0.296	0.273
3	15.90	3.567	0.183	0.8808	1.018	0.1595	0.376	0.625
4	23.67	7.075	0.161	0.9972	0.911	0.0781	0.301	1.210
5	21.21	5.854	0.183	0.9816	0.945	0.1581	0.017	0.686
6	15.34	2.855	1.303	0.8881	0.938	0.4478	0.041	0.330
7	23.51	6.897	1.454	0.8579	0.622	0.3002	0.058	0.299

* Omitted because of insufficient material.

iron in all the plants was greater in the roots than in the stems and the blades. The percentages of iron in the roots of series 4 and 5 were greater than in 6 and 7.

The results of the ash analyses, recalculated to show the total amounts per plant in grams, are presented in tables XVII and XVIII. Table XVII

TABLE XVII

AVERAGE TOTAL AMOUNT OF ASH CONSTITUENTS IN PLANTS HARVESTED NOVEMBER 20, EXPRESSED IN GRAMS; RECALCULATED FROM TABLE XV

SERIES	AVERAGE TOTAL DRY WEIGHT (OVEN DRY)	ASH	SI	K	P	CA	MG
Blades	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	66.3	3.41	0.044	1.180	0.238	0.185	0.113
2	66.6	2.81	0.041	0.636	0.264	0.274	0.256
3	36.3	1.69	0.032	0.196	0.242	0.182	0.196
Roots (air dry)							
1	23.7	5.915	1.898	0.421	0.142	0.126	0.073
2	28.7	5.000	1.307	0.213	0.129	0.219	0.135
3	11.2	3.199	1.052	0.043	0.084	0.058	0.031
4	5.8	1.840	0.662	0.017	0.042	0.032	0.014
5	6.0	2.029	0.659	0.020	0.051	0.037	0.016

shows that the blades of series 1 and 2 were nearly the same in dry weight per plant; that series 1 had a higher total ash content than series 2 chiefly because of the higher amount of potassium in series 1 than in 2; that silicon was very nearly equal in the two series; and that phosphorus, calcium, and magnesium were all present in greater amounts in series 2 than in series 1. Even series 3, the blades of which had half the dry weight of series 1, contained more phosphorus and magnesium and nearly the same amount of calcium as series 1. The roots of series 2 contained more calcium and magnesium than those of series 1, but the roots of series 3 were lower in all the ash constituents studied.

Table XVIII shows that at the age of seven and one-half months the total amounts of ash constituents in the plants depended more upon the size of the plant than upon the percentage of potassium. Both the stems and roots of series 2 weighed less than those of series 1, but the stems of series 2 had more silicon, calcium, magnesium, and sodium than series 1; and the roots of series 2 had more ash, silicon, phosphorus, calcium, magnesium, sodium, and iron than in series 1. It was therefore by no means general that the plants deficient in potassium contained greater total amounts of ash constituents per plant than the controls, probably because the former

TABLE XVIII

AVERAGE TOTAL AMOUNTS OF ASH CONSTITUENTS IN PLANTS HARVESTED APRIL 27, EXPRESSED IN GRAMS; RECALCULATED FROM TABLE XVI

SERIES	AVERAGE TOTAL DRY WEIGHT	ASH	Si	K	P	Ca	Mg	Na	Fe
Blades (oven dry)	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	199.8	14.7	0.235	4.345	1.019	1.090	0.859	0.028	0.044
2	263.0	20.7	0.247	4.737	1.405	2.214	1.478	0.097	0.045
3	68.6	3.9	0.071	0.497	0.448	0.191	0.354	0.021	0.037
4	14.4	0.9	0.019	0.051	0.115	0.031	0.092	0.005	0.004
5	14.9	1.0	0.026	0.074	0.118	0.036	0.081	0.004	0.008
6	124.8	8.1	0.188	2.203	0.658	0.348	0.339	0.031	0.039
7	105.8	7.3	0.017	2.618	0.525	0.279	0.274	0.026	0.067
Stems (oven dry)									
1	299.4	13.47	0.357	4.50	1.27	0.347	0.547	0.059	0.059
2	268.9	8.55	0.425	1.17	0.97	0.513	0.729	0.347	0.043
3	35.9	1.07	0.037	0.12	0.15	0.053	0.091	0.051	0.023
4	4.9	0.18	0.006	0.01	0.03	0.008	0.012	0.012	0.012
5	6.5	0.27	0.009	0.02	0.04	0.010	0.019	.....	.....
Roots (air dry)									
1	108.5	13.17	2.06	0.977	0.848	1.075	0.232	0.089	0.253
2	106.8	15.89	3.18	0.377	0.867	1.339	0.304	0.316	0.291
3	27.8	4.42	0.99	0.051	0.245	0.283	0.044	0.104	0.174
4	8.2	1.94	0.58	0.013	0.082	0.075	0.006	0.025	0.099
5	11.2	2.40	0.66	0.021	0.111	0.106	0.018	0.002	0.077
6	47.6	7.30	1.36	0.620	0.423	0.446	0.213	0.019	0.157
7	40.5	9.52	2.79	0.589	0.347	0.252	0.121	0.023	0.121

were generally smaller. However, the fact that they did contain more in the places just mentioned increases the importance of the differences in percentage and leads to the conclusion that the absorption of potassium tends to decrease the absorption of such elements as phosphorus, calcium, magnesium, and iron.

Discussion

The economic importance of physiological studies of potassium deficiency in sugar cane lies in the effects upon the growth, resistance to diseases, and quality of the juices exerted by potassium. A survey of the literature dealing with sugar cane shows that there is no real agreement regarding the value of potash fertilization, inasmuch as the results of some experiments indicate a response while others do not. No attempt will be made to cite all the pertinent references, but a few representative ones will be mentioned as illustrations. The importance of potassium in increasing growth and yield of cane has been discussed by STEWART and VERRET (**68**) and by MOIR (**53**), who stress its necessity in the early growth of cane.

NAQUIN (**54**) has found that there is a greater response to potash fertilization in hollows than on knolls or in thin soil. AYRES (**8**) found that cane in some crops used well over 800 lb. K_2O per acre, illustrating the tremendous demand made by cane for potash. MCGEORGE (**50**), on the other hand, states that the majority of Hawaiian soils do not respond to potash fertilization.

Among those reporting gains in juice quality resulting from the application of potassium, the following may be mentioned: AGEE (**1**), ALEXANDER (**3, 4**), HARRISON (**27**), and PRATT (**61**). FERRIS (**19**) found no increase in yield due to the addition of potassium. VERRET (**71**) reported that there was no effect whatever on the quality ratio from the use or omission of phosphoric acid or potash or both. According to GEERLIGS (**21, 22**), generally a juice of low purity is highest in potassium, while juices rich in sucrose contain less potassium, and those varieties which make the most sugar generally have the least potassium. GEERLIGS concluded that many other factors have a greater effect upon quality than potash. That this condition of non-conformity of results of potash studies still exists is shown by a recent report by AGEE (**2**), in which the results of several experiments are summarized. When one considers the sources of error in field experiments as at present conducted, there is no wonder that the results are not always in agreement. Of course the response from applications of potash depends upon the amount of potassium already in the soil and many other factors. MOIR (**53**) stresses the balance as important, stating that the application of plant foods aids crop quality when the element lacking is supplied, and continues to do so until some other element becomes limiting or until one is added in too large amount.

The importance of potassium in increasing resistance to disease in plants is well known. Sugar cane is no exception. LEE (**43**) and LEE and MARTIN (**44**) report that potassium aids in increasing resistance toward eye spot. MOIR (**53**) suggests that the increase in cutin due to potassium found by HARTT (**28**) points to the effect of potassium in lessening damage due to cane diseases such as eye spot and brown stripe. He also states that the large cavities in the cortex of the roots and the poor root hairs in the potassium-deficient plants reported by HARTT may suggest one possible cause of the root failure complex or Lahaina disease. The evidence is inconclusive regarding a relationship between a lack of potassium and brown stripe disease. HANCE (**26**) by spectroscopic analysis found that cane plants susceptible to brown stripe were deficient in barium, potassium, magnesium (or copper), and cadmium (or zinc). SHEPARDSON (**64**), however, found no apparent correlation between brown stripe and potassium, but suggested further studies concerning silicon and that disease. In gen-

eral there is need for more investigation of the relation between potassium and disease resistance in sugar cane.

The preceding brief review illustrates the need for more fundamental physiological studies of potassium in sugar cane.

Although similar in plan and purpose, the present studies are not absolutely comparable with those carried on in Chicago (**28**), for several reasons. In the Chicago experiment the variety used was Louisiana Purple (Cheribon), while H 109 was used in the Honolulu studies. The growing conditions also differed considerably. In the former investigation the plants were grown during the winter and spring in Chicago and during much of the time the light conditions were poor, although supplemented by electricity. In Honolulu the length of day and amount of light were more nearly uniform and better adapted for the growth of cane. The temperature also differed, the Chicago greenhouse being kept around 90° F., whereas in Honolulu the temperature at times reached 110° F. Because of the differences in light and temperature, the growth rate of the plants in the present investigation was considerably greater than that of the first study. The nutrient solutions used in Honolulu were less concentrated than those used in Chicago but were renewed more frequently. Also silicon, manganese, and titanium were used in these studies and not in the former. Iron was added as ferric phosphate in the former, whereas ferrous sulphate was used in the latter investigation. Undoubtedly there were other differences in the growing conditions of the two experiments. Secondary growth was purposely discouraged in the Chicago experiment, whereas in Honolulu the secondary shoots were allowed to develop. The writer thus agrees with the criticism of VAN DEN HONERT (**70**) that the plants in Chicago were grown under unfavorable light conditions. Notwithstanding these differences in variety, light, temperature, growth rate, and nutrient solutions, similar symptoms of potassium deficiency occurred in both and it is felt that broad comparisons are justified between the two investigations.

Evidence of the difference in the growth rates of the plants grown in Chicago and Honolulu is given in table XIX. This shows that in one month the main stalks of the plants grown in Honolulu were nearly as tall as those in Chicago in about five months, when the lengths of the longest leaves are compared. The first symptoms of potassium starvation developed in Honolulu within one month after watering with the nutrient solutions was begun, while four months elapsed in Chicago before deficiency symptoms appeared. While other factors no doubt contributed to these differences in growth and development of symptoms, yet it is felt that one of the most important causes was the amount of light. In this connection mention should be made of an unpublished study conducted by Miss MARJORIE

BODWELL in 1931, at that time a student with the writer at Connecticut College. Miss BODWELL grew buckwheat and soy bean plants with and without potassium under several intensities of light. The plants in the most intense light were the first to exhibit symptoms of potassium starvation, the others following in the order of decreasing intensities of light.

TABLE XIX

COMPARISON OF GROWTH: CHICAGO AND HONOLULU

SOLUTION NO.	CHICAGO STUDIES			HONOLULU STUDIES			
	K	LENGTH OF LONGEST LEAF		LENGTH OF LONGEST LEAF		K	SOLUTION NO.
		1 MO. 15 DAYS	5½ MO.	1 MO. 5 DAYS	3½ MO.		
	p.p.m.	*cm.*	*cm.*	*cm.*	*cm.*	*p.p.m.*	
1	703.8	88.9	170.2	140.7	198.6	87.9	1
4	39.0	67.3	143.5	147.1	198.2	39.0	2
3	3.9	68.9	151.1	133.7	160.5	3.9	3
2	0.0	71.7	139.7	125.8	135.9	0.0 (+Na)	4
				120.3	136.9	0.0 (–Na)	5

The effect of light upon the absorption and utilization of potassium by plants has been studied by several workers. JOHNSTON and HOAGLAND (**39**) have given evidence that plants absorb more potassium during the day than at night and suggested that the energy of light may be an important factor governing the absorption of nutrient ions by plants. NEMEC (**56**) grew rye under colored glass bell jars and found that plants under green jars absorbed less potassium than those in full sunlight, while those under violet and red jars contained more potassium than those in sunlight. NIGHTINGALE (**57**) refers to STOKLASA who found that sugar beet seedlings in darkness when supplied with potassium and sugar accumulated much more dry matter than plants in the dark lacking potassium but supplied with sugar; while in the light, the amount of sugar absorption was only a little higher in the plants supplied with potassium than in those lacking it. It was therefore suggested that potassium to some extent assumes the rôle of light. A connection between light and potassium seems substantiated. Since in the present studies equal amounts of potassium were absorbed by sugar cane plants during twelve hours of darkness and twelve hours of daylight (table XII), it would seem that light must affect the utilization of potassium by sugar cane rather than its absorption. Whether this may be due to the photoelectric properties of potassium or is merely a matter of limiting factors will be discussed in the second paper of this series.

Absorption of potassium

The amount of potassium absorbed probably depends more upon its amount and availability in the soil than upon the rate of its utilization within the plant, since considerable evidence has been given by Bartholomew and Janssen (**9**) indicating that plants may indulge in a luxury consumption of potash. Undoubtedly the age of the plant is also an important factor influencing the absorption of potassium, particularly when young. Evidence of this is given in table XI, showing that one month after watering with the nutrient solutions was begun, the plants of series 1 and 2 were absorbing potassium at the same rate. The plants were about the same size at that time, no symptoms of potassium deficiency having yet appeared in series 2. As is shown in figure 15, early in November, the plants of series 1 definitely surpassed those of series 2 in growth. It is interesting that on November 13 and 14, series 1 showed the ability of absorbing double the amount of potassium absorbed by series 2, according to table XII. It would seem that when supplied with 39–88 p.p.m. potassium, the amount absorbed during the first month is limited by the size of the plant, probably in particular by the size of the root system; but when there is 3.9 p.p.m. potassium or less, the amount of potassium is the limiting factor, and potash starvation symptoms develop earlier. Inasmuch as the absorption of potassium seems to be limited by the size of the young plant, it is apparent that large applications of potash in the field during the first month may not be economical.

André and Demoussy (**6, 7**) have stressed the importance of the mobility of potassium. The absorption of considerable amounts of potassium may decrease the amounts of certain other elements absorbed, which may offer a partial explanation for the depressing effect of potassium upon the growth and juice quality of cane which has been mentioned by Agee (**2**). In the present study, little evidence of a depressing effect upon growth was obtained. Three weeks after starting the plants in the nutrient solutions, the plants of series 2 slightly surpassed those of series 1 in growth, as shown in table II. This difference held also in nine weeks for certain measurements (tables III–V), but at that time the average height of the highest dewlap of the plants of series 1 was higher than that of series 2; and from then to the end of the experiment the growth of the plants of series 1 steadily surpassed that of series 2, as shown in figure 15.

Sugar cane plants deficient in potassium were found to absorb more of certain other elements on the percentage basis than did the controls, a condition similar to that studied in other plants by various investigators: Anderson *et al.* (**5**), Davis (**16**), Fonder (**20**), Ginsburg (**24**), Hoagland (**32**), Owen (**59**), and others. Two months after starting the plants in the nutrient solutions, the potassium-deficient plants were absorbing more

silicon, phosphorus, calcium, and magnesium than the controls, as shown by table XV. That this was a real difference in amount is shown by the fact that the plants of series 4 and 5 had the largest percentage of total ash, particularly in the roots, and is also shown by the greater total amounts of ash constituents in the plants deficient in potassium than in the controls, found in some of the series, as shown in tables XVII and XVIII. This difference in absorption did not continue to the end of the experiment, as at the time of final harvest the potassium-deficient plants had the lowest percentage of total ash in the tops, although they still had the highest percentage in the roots. The absorption of phosphorus continued to be greater in the plants starved for potassium to the end of the experiment, which may have an important bearing upon the activity of amylase, as will be mentioned in the second paper in this series. The final percentages of calcium and magnesium varied irregularly in the different parts of the plants, except that calcium was highest in the blades of the plants of series 1 and 2.

Determinations were also made of the amounts of iron and sodium in the plants harvested in April. The percentages of iron in the blades, stems, and roots of the plants deficient in potash were found to be higher than in the controls, showing that a deficiency in potassium leads to the absorption of a greater amount of iron. Sodium was lower in amount than potassium in the blades and the stems of all the plants, even those supplied with more sodium than potassium. These comparisons are taken from table XVI.

The addition of potassium to the potassium-deficient plants resulted in a rapid absorption of that element and a considerable increase in growth. The steps in recovery outlined in the results were similar to those obtained in the field, according to Moir (personal communication). Table XVI shows that in some instances the plants of series 6 and 7 contained higher percentages of potassium than did the controls. To a certain extent this may be due to the fact that the absorption of potassium was more rapid than the resulting growth. The plants of series 7 contained more potassium than did those of series 6 in all organs except the dead leaves. This higher percentage in series 7 led to a considerably greater growth, series 7 surpassing series 6 to a marked degree during the latter part of the experiment. The addition of potassium to the plants of series 6 and 7 also decreased the percentages of phosphorus and magnesium in those plants.

The effect of the addition of potassium upon the distribution of iron within the plant is important. Table XVI shows that the plants of series 6 and 7 had considerably lower percentages of iron in the roots than those of series 4 and 5, indicating that the addition of potassium to the former beginning in January had the important effect of decreasing the percentage of iron in the roots as a part of their recovery from potassium deficiency. Table XVI also shows that the stems of the plants deficient in potassium

contained larger percentages of iron than the controls, whereas the plants of series 6 and 7 contained nearly the same amount in the stems as the controls. It is evident that the addition of potassium to the potassium-deficient plants resulted in a decrease in the percentage of iron within the roots and stems. That this was not entirely the effect of a greater distribution of the iron within the larger stems is shown by the relative amounts of iron in the blades and stems of the plants of series 6 and 7. Table XVI shows that the percentages of iron in the blades of series 6 and 7 were nearly double or treble the percentages in the stems of those plants, whereas in series 1–4 the percentages of iron in the blades were equal to or less than the percentages in the stems. It seems that the addition of potassium to the plants starved for that element caused some of the iron which had accumulated in the stems to pass up into the blades. This may be explained by assuming that the addition of potassium increased the turgidity of the leaves and thus increased the speed of the upward movement of the transpiration stream and the mass movement of the iron. That potassium affects turgidity is well known, having been established by COPELAND (**13**) as early as 1897. Evidence that the rate of transpiration is affected by the supply of potassium was obtained in a test using the cobalt chloride paper method, in which equal shades of pink were developed in 55 seconds on a blade of one of the plants of series 1, and in four minutes on a blade of one of the plants of series 3, these blades having similar exposures.

To summarize, several generalizations may be offered regarding the absorption of potassium by the sugar cane plant, from the studies reported in this paper:

1. Since equal amounts of potassium were absorbed by day and by night, the absorption of potassium does not seem to be affected by light.

2. With the exception of the first three or four weeks, the amount of potassium absorbed is directly proportional to the amount supplied, within the limits of this experiment.

3. During the first month the absorption of potassium is limited by the size of the plant.

4. Cane plants deficient in potassium absorb more phosphorus and iron, at least during the first seven months of their growth. They also absorb more calcium, magnesium, and silicon during the first two or three months.

5. The addition of potassium to plants deficient in that element results in a rapid absorption of potassium, a decrease in the intake of phosphorus and magnesium, and causes some of the iron which had accumulated in the stems to pass up into the blades.

MIGRATION OF POTASSIUM

There was a greater percentage of potassium in the blades than in the stems, and the stems in turn were higher in potassium than were the dead

leaves (table XVI). In every series the percentage of potassium in the dead leaves collected in April was not only lower than in the blades harvested at the same time but also lower than in the blades of the November material. Therefore the potassium migrated from the dying leaves to the living top of the sugar cane in this experiment, as has also been shown by BONAME (**10**). BARTHOLOMEW and JANSSEN (**9**), JAMES (**36**), JANSSEN and BARTHOLOMEW (**37**), NIGHTINGALE and coworkers (**57**), and others have mentioned this in connection with other plants. This migration is against the diffusion gradient.

If the percentage of potassium in the dead leaves is subtracted from the percentage in the blades (table XVI), a rough estimate of the amount of migrated potassium is obtained. Then if the ratio is taken of the percentage of migrated potassium to the percentage of potassium left in the dead leaves, the results in table XX are obtained. This shows that the relative amounts of migration are in this order: $2 > 3 > 5 > 7 > 4 > 1 > 6$. If this is compared in a similar manner with the percentages of the following in blades: total ash, K, Si, P, Ca, Mg, Fe, Na, and H_2O, it is found that there is no relation between any of the ash constituents studied and the migration of potassium. The moisture content decreased in this order: $1 > 6 > 7 > 4$ and $5 > 3 > 2$. Omitting the plants of series 6 and 7, it is found that the migration of potassium occurred in this order: $2 > 3 > 5 > 4 > 1$. The water content decreased in the order: $1 > 4$ and $5 > 3 > 2$. Possibly the plants of series 6 and 7 should be considered separately inasmuch as their treatment was changed during the course of the experiment. Of the two, series 6 had the greater moisture content and the lower migration of potassium while series 7 had the greater migration of potassium and the lower percentage of water. The conclusion seems inevitable that the greatest migration of potassium occurred in the blades having the lowest water percentage, and that there was no relationship between the amount of migration and the percentage

TABLE XX

RELATIVE AMOUNTS OF MIGRATION OF POTASSIUM

SERIES	% POTASSIUM MIGRATED ÷ % POTASSIUM LEFT IN DEAD LEAVES
1	0.71
2	7.4
3	3.5
4	0.73
5	2.0
6	0.4
7	1.1

of potassium in the plant. In other words, migration did not occur most readily in those plants which needed it most; therefore the translocation of potassium is not an adaptation in potassium-deficient plants, but occurs there more than elsewhere only when those plants at the same time have the lowest moisture content. The suggestion made by PENSTON (**60**) is pertinent, that migration is possibly related to age rather than to a deficiency in potassium. It is probably an exception in the present study that the plants of series 2 contained less water than those of series 3, 4, or 5, possibly explained by their greater size and the fact that for some undetermined reason it was practically impossible to keep the plants of series 2 adequately supplied with water, as they required even more than series 1. It is probably more generally true that the moisture percentage is directly proportional to the amount of potassium supplied, since several investigators (**11, 28, 35, 38, 72**) have reported lower moisture contents or higher percentages of dry matter in potassium-deficient plants; hence the migration of potassium may in general occur most in the plants which benefit most by that condition. It is felt that statements in the literature regarding such translocations often verge on the teleological, when it is implied or stated that plants deficient in potassium can transfer the little potassium they contain from a place where it is no longer in use to where it is needed. The results herein reported show that this is not an adaptation in potassium-deficient plants, since it occurred most readily in the plants of series 2, which contained considerably more potassium than the plants of series 3, 4, or 5, and occurred equally well for series 1 and 4. DOWDING (**18**) also found that a translocation of potassium occurs within the embryonic cone during bud elongation in the spruce, and those plants were not deficient in potash.

Probably several factors are involved in the migration of potassium. In leaves it may simply be a balance of forces between the transpiration of the plant as a whole and that of the lower leaf. When the lower leaf contains very little water the pull of transpiration from above readily overcomes the pull of the individual dying leaf, but when the lower leaf contains a considerable amount of water, such is not the case. The failure of the development of the abscission layer in plants in extreme potassium deficiency, found in cane by NAQUIN (**55**), who stated that in potassium deficiency the dead leaves adhere to the stalk, and reported in the tomato by LIPMAN (**46**), is another important factor. Without the usual abscission layer the leaf would tend to remain longer on the plant, thus allowing migration. KOSTYTSCHEW and ELIASBERG (**42**) were among the first to find that potassium can be extracted from plant tissues *in toto* by water. Since JANSSEN and BARTHOLOMEW (**37**) and others have also found that most of the potassium in plants is soluble in water, it seems that potassium is

present in a readily movable form. Undoubtedly other factors are involved, because the situation is not always so simple. Potassium not only migrates into the upper leaves, but also into fruits and to the meristematic tips of roots as reported by LIPMAN (**46**). If electric currents of mitotic origin similar to those found in wheat plants by COLLA (**12**) are of general occurrence, possibly these might aid in explaining the migrations of potassium to meristematic regions. The suggestion proposed by MCGEORGE (**52**) that bases may be absorbed by lignin and displaced by other bases or by hydrogen, and that this interchange may play a part in ionic movement through plant tissue, may contribute to the explanation of the mechanism of the translocation of potassium. In short, the forces involved in the migration of potassium from the older regions of plants to young developing portions possibly include transpiration, base exchange, and electrokinetic phenomena.

Of the ash constituents studied, potassium was the only one which exhibited the ability to migrate. Table XVI shows that the dead leaves of all the plants had a higher percentage of total ash than had the green blades. The silicon, phosphorus, calcium, magnesium, iron, and sodium varied irregularly in most of the groups, being greater in amount in the dead leaves than in the blades, with occasional exceptions. This is to be expected, since with the exception of sodium the elements mentioned are known to enter into organic compounds, either in wall material or protoplasm; potassium exists for the most part in simple form. Another factor involved is the greater mobility of potassium as compared with the other elements, which has been stressed by ANDRÉ and DEMOUSSY.

IRON TOXICITY

An interesting controversy has occurred in Java as to whether certain symptoms in sugar cane may be attributed to potassium deficiency or to iron toxicity. In the condition known as Kalimati disease (so called from the name of the principal plantation where it occurs), the symptoms are very similar to those of potassium deficiency and the addition of potassium has been found to overcome the trouble. WILBRINK (**73**) thinks that Kalimati disease is caused by potassium deficiency. KONINGSBERGER (**40, 41**), however, is of the opinion that the disease is due to iron toxicity, which occurs particularly with ferrous iron when ammonium nitrogen is used instead of nitrate nitrogen. He thinks that the disease is overcome by the addition of potassium either because potassium counteracts the injurious action of iron or because it may stimulate nitrification. That the Kalimati disease is overcome by the addition of potassium is insufficient ground for calling the disease potassium deficiency, according to KONINGSBERGER, who feels that that is no better than to speak of "quinine deficiency" in the case of malaria.

Whether one agrees with WILBRINK or with KONINGSBERGER, it is interesting that a toxic effect of iron, which can be overcome by the addition of potassium, occurs in both Kalimati disease and potassium deficiency. HOFFER (**34**) reported that the addition of potassium to corn plants having accumulations of iron at the nodes cleared up that condition but the accumulation of aluminum was not affected. HOFFER found the vessels of plants deficient in potassium clogged with iron, and these vessels became clear upon the addition of potassium. He pointed out that this accumulation causes the breakdown, either by precipitation or by coagulation, of the cell contents of certain joint tissues, interfering with translocation. Furthermore, HARVEY (**30**) stated that in maize grown in acid soil there may be a deposit of Fe_2O_3 in the vascular tract sufficient to interfere with translocation, and that firing of the leaves may be observed under such conditions. MCGEORGE (**51**) found that both potassium and phosphorus aid in the distribution of iron and that any growth-retarding factor may induce accumulations of iron and aluminum in the nodes of cane. In the present study iron was found to be higher in the potassium-deficient plants than in the controls, particularly in the roots and stems. Iron occurred especially at the nodes of the plants deficient in potassium but not of the controls.

Either because of this nodal accumulation of iron or on account of an upset in the synthesis of proteins resulting in abnormal protoplasm in the sieve tubes and companion cells, or for both reasons, a necrosis of the phloem occurred in the plants deficient in potassium. The derangement in the formation of proteins will be discussed fully in the second paper of this series. In the previous study (**28**) a little evidence was obtained of derangements of the phloem in the plants deficient in potassium. Reference to the section dealing with microchemical results will show that further evidence of phloem necrosis in the plants deprived of potash has been offered in the present study. The evidence consisted of a dark discoloration of the bundles of the stems and of both sieve tubes and companion cells of the bundles of the midribs. These discolorations were noted as early as the latter part of October. Furthermore, it has recently been found by MARTIN (**49**) that similar discolorations of the bundles in cane stems occur throughout the Hawaiian Islands, and there is some evidence that they are related to potash deficiency. This condition has been termed "internal stalk necrosis."

These accumulations occur more at the nodes than at the internodes, possibly because of the anastomosis of the bundles in the former location. Perhaps another factor involved in the nodal accumulations of iron is the tannin content which, according to HALDEN (**25**), is particularly large in the nodes. It would seem that the insolubility of iron tannate must play a

part in the deposition of iron at the nodes, although how the addition of potassium could clear up the iron tannate is difficult to explain. X-ray photographs of nodal accumulations in sugar cane have been published by CRUTCHFIELD (**14**).

Evidence is presented in table XVI showing that the addition of potassium to plants deficient in that element resulted in a considerable decrease in the percentage of iron in the roots and stems and a slight increase in the blades, leading to the conclusion that potassium caused some of the iron to pass up from the stems to the blades. As mentioned before, this was possibly due to an increase in the transpiration rate; hence the upward movement of the transpiration stream following the addition of potassium. The fact that the transpiration stream of potassium-deficient plants is so sluggish as to permit the lodging of sufficient iron and aluminum to clog the vessels and yet strong enough to pull out the remaining potassium in the lower leaves is possibly explained by the differences in the mobilities of these elements. Because of the tendency to use these nodal accumulations of iron as a symptom of potassium deficiency, although they have been found to occur in both calcium and phosphorus deficiency (**52**) as well as increased soil acidity (**30**), the need is felt for further study of this question. Because the plants which contained the largest percentages of iron did not have as much as some of MCGEORGE'S (**51**) plants which gave good growth (5.1 per cent. iron in the roots of plants 4 compared with 21.8 per cent. iron in one of MCGEORGE'S plants, both on the ash basis), the results of the present study support the view advanced by MCGEORGE that the toxicity of iron in sugar cane is due to its unequal distribution rather than to its total concentration.

One of the conditions which must be taken into account in a consideration of potash starvation therefore is iron toxicity. One of the most important derangements in potassium deficiency to which iron toxicity contributes is the disturbance in the translocation of the sugars and nitrogenous compounds, which will be discussed in the second paper of this series.

CAN SODIUM REPLACE POTASSIUM?

The question of the possibility of the substitution of sodium for potassium in the nutrition of plants, which was settled temporarily by NOBBE, SCHROEDER, and ERDMAN (**58**) in 1871, has been reopened in recent years. Several investigators (**29, 31, 66**) report at least the partial substitution of sodium, whereas others, for example REED and HAAS (**62**), conclude that there is no evidence that sodium can replace potassium.

To study this question with cane, the plants of series 5 were supplied with a nutrient solution lacking both sodium and potassium. Very little difference was found between the plants of series 4 (supplied with sodium)

and those of series 5 (deprived of sodium) in either growth or external appearance. Their similarity in appearance is shown in figures 10 and 11, 19 and 20, 26 and 27. Tables II–VI, VIII, and IX show that where there was a difference in size between series 4 and 5, the plants of series 5 were generally the larger, indicating that the sodium may have exerted a somewhat depressing effect. Figure 15 shows that the stems of these plants grew at about the same rate, their curves crossing at several points. The plants of series 5 showed a better ability to recover upon the addition of potassium than series 4, which perhaps was due to the greater supply of calcium in solution 5, possibly leading to a better absorbing system.

The percentages of sodium in the ash of all the plants are given in table XVI, from which it will be seen that the plants receiving sodium in the nutrient solutions contained more than the others; but except in the stems, the percentage of sodium in the plant was not proportional to the amount supplied.

All the blades contained higher percentages of potassium than of sodium, even those supplied with more sodium than potassium. This was true also for the stems and for the roots, except in series 3 and 4. The smaller amounts of potassium in the solutions were evidently absorbed more readily than the larger amounts of sodium. Similar results were reported by André and Demoussy (**6, 7**), who found that plants supplied with more sodium than potassium absorbed more potassium than sodium, which they explained by the greater mobility of the element potassium.

The results of the present study, therefore, lead to the conclusion that sodium does not even partially replace potassium in the nutrition of sugar cane, but may possibly exert a slightly depressing effect upon growth.

Summary

1. Sugar cane plants, variety H 109, grown in quartz sand cultures, were supplied with varying amounts of potassium as follows: 87.9 p.p.m. K, 39 p.p.m. K, 3.9 p.p.m. K, no K + Na, no K, no Na.

2. This paper deals with the effects of varying amounts of potassium upon the growth of sugar cane and upon the absorption and migration of the constituents of the ash. The following paper reports the results of determinations of the activity of the enzymes invertase, amylase, and ereptase; also analyses of total and amino nitrogen, reducing sugars, and sucrose; and the hydrogen ion concentration, titratable acidity, and titration curves of the juices expressed from the leaves, stems, and roots.

3. Symptoms of potassium starvation began to appear three weeks after watering with the nutrient solutions was begun.

4. The symptoms of potassium deficiency which developed were similar to those which occurred in the previous study (**28**): depressed growth of the plant as a whole, discoloration of the leaves, and dieback of the leaf tips.

5. The development of red midribs, which occurred on the plants deficient in potassium, is shown to be a secondary rather than a primary symptom of potash deficiency, developing when phloroglucin and sufficient sulphate occur simultaneously within the plant. The source of phloroglucin suggested is the decomposition of tannic acids, a possible result of potassium deficiency. The sulphate came from an excess of ferrous sulphate which was used as the source of iron.

6. The growth of the plants was proportional to the amount of potassium supplied.

7. Because the plants totally deprived of both sodium and potassium equaled or surpassed those supplied with sodium but no potassium, the conclusion is drawn that sodium does not even partially replace potassium in the nutrition of sugar cane.

8. The addition of potassium to plants deficient in that element resulted in a rapid absorption of potassium and a considerable increase in growth. The absorption of phosphorus and magnesium was decreased in these plants.

9. Plants previously deprived of both sodium and potassium recovered upon the addition of potassium more rapidly than did those previously deprived of only potassium.

10. During the first month the absorption of potassium was limited by the size of the plant, whereas after the first month the amount of potassium absorbed was directly proportional to the amount supplied.

11. Since equal amounts of potassium were absorbed by day and by night, the absorption of potassium was found not to be affected by light.

12. No evidence was obtained showing that sugar cane can absorb potassium through the leaves.

13. The sugar cane plants deficient in potassium absorbed more phosphorus and iron than the controls, at least during the first seven months of their growth. They also absorbed more calcium, magnesium, and silicon during the first two or three months.

14. The toxicity of iron in sugar cane is due to its unequal distribution rather than to its total concentration, a conclusion agreeing with that reached by McGeorge.

15. Nodal accumulations of iron were found in the plants starved for potassium.

16. The addition of potassium to plants deprived of that element caused some of the iron which had accumulated in the roots and stems to pass up into the blades, possibly due to an increase in turgidity of the leaves and in the upward movement of the transpiration stream.

17. Brown discolorations of the sieve tubes and companion cells in the midribs of the plants deficient in potassium indicated a necrosis of the phloem.

18. The control plants had a higher moisture content in both stems and blades than had the plants deprived of potash.

19. The percentage of potassium within the plants was directly proportional to the amount supplied in the nutrient solutions. A similar relationship held for sodium only in the stems.

20. With the exception of the roots of the plants of series 3 and 4, all the plants contained higher percentages of potassium than sodium in all organs, even those plants supplied with more sodium than potassium.

21. The ash analyses showed that potassium migrated from the dying leaves to the living top of sugar cane, and that no other elements studied did so.

22. The greatest migration of potassium occurred in the blades having the lowest moisture percentage. There was no relationship between the amount of migration and the percentage of potassium. Therefore the translocation of potassium is not an adaptation in potassium-deficient plants but occurs there more than elsewhere only when those plants at the same time have the lowest moisture content.

23. Some evidence is presented indicating that the transpiration of the plants deficient in potassium was less than that of the controls.

EXPERIMENT STATION, H. S. P. A.
HONOLULU, T. H.

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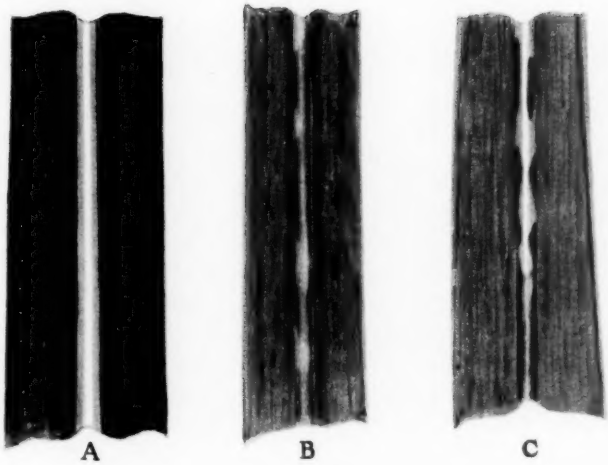
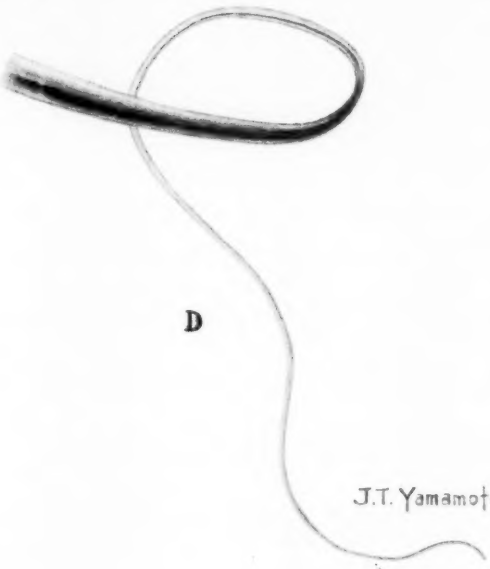
EXPLANATION OF COLOR PLATE

Color symptoms of potassium deficiency in sugar cane: *A*, typical leaf from control plant; *B*, *C*, and *D*, leaves from plants deficient in potassium. *B* shows the color of a midrib soon after it has become red, while *C* shows the darkening of the discoloration as the leaf ages.



A B C



D

J.T. Yamamoto

SOME EFFECTS OF POTASSIUM UPON THE AMOUNTS OF PROTEIN AND AMINO FORMS OF NITROGEN, SUGARS, AND ENZYME ACTIVITY OF SUGAR CANE[1]

CONSTANCE ENDICOTT HARTT

(WITH ONE FIGURE)

Introduction

This paper reports the results of determinations of the enzymes invertase, amylase, and ereptase; analyses of total and amino nitrogen, reducing sugars and sucrose; and the hydrogen ion concentrations, titratable acidity, and titration curves of the juices expressed from the leaves, stems, and roots of plants grown with varying amounts of potassium.

METHODS.—Details of the care of the plants during growth and of the methods employed in harvesting them have been presented in the preceding paper (**26**).

Invertase, amylase, peptase, and ereptase, and total and reducing sugars were determined by the methods used in the studies reported in 1929 (**24**). The titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ (**55**) was also used for the detection of peptase.

Total nitrogen was determined by the Kjeldahl method, while the Van Slyke method was employed for the estimation of amino nitrogen. The nitrogen determinations were performed by the Chemistry Department of this Station.

Results

1. PHYSICO-CHEMICAL STUDIES OF EXPRESSED SAP

Determinations of the hydrogen ion concentration, titratable acidity, and titration curves were performed using two representative plants of series 1 and 3, harvested December 4, 1931, eleven weeks after starting the plants in the nutrient solutions. The results of the electrometric determinations of the hydrogen ion concentration of the sap expressed from frozen tissues are presented in table I. These data show very little difference between the hydrogen ion concentration of the controls and that of the plants deficient in potassium, the former being slightly more acid than the latter, which is the reverse of the results obtained by REED and HAAS (**40**).

Because of the dark color of the juices, it was necessary to dilute 1 cc. of juice with 20 cc. distilled water before titration. N/5 NaOH was used, with phenolphthalein as indicator. The results are given in table I. This shows that the blades of series 1 were a little less acid than those of series

[1] See footnote 1 of preceding paper.

TABLE I

pH AND TITRATABLE ACIDITY OF EXPRESSED SAP

SERIES	pH	N/5 NaOH TO NEUTRALIZE 1 CC. JUICE
		cc.
Blades		
1	5.26	0.32
3	5.32	0.49
Stems		
1	5.17	0.4
3	5.34	0.3
Roots		
1	5.58	0.09
3	5.64	0.09

3, while in the stems the reverse held, and the roots showed no difference.

By adding varying amounts of sulphuric acid and sodium hydroxide to the expressed sap and determining the hydrogen ion concentration electrometrically, the titration curves were obtained. These are shown in figure 1. A slight change in pH upon addition of acid or alkali indicates that the juice is well buffered, while a greater change means a poorly buffered juice. The graphs show that the blades of series 3 seemed to have a slightly better buffer system than those of series 1; that the buffer systems of the stems were very much alike; but that with roots, the plants of series 1 had a slightly better system on the acid side but those of series 3 were better on the alkaline side. On the whole there was very little difference in the way they reacted to additions of acid and alkali. It would seem, therefore, that differences in hydrogen ion concentration, titratable acidity, and buffer systems were insufficient to explain the results of the enzyme determinations here reported.

2. Analytical data

The results of the nitrogen determinations are presented in tables II and III.

In November, there was a higher percentage of amino nitrogen and lower percentage of protein nitrogen in the plants of series 3 than in the controls, while the total nitrogen remained about the same. These differences were more conspicuous in the blades than in the stems. In April, higher percentages of amino nitrogen, protein nitrogen, and total nitrogen were found in the blades of the plants deficient in potassium, while in the stems the opposite relationship was found. When the total amounts of nitrogen contained in the entire plants are calculated, the results presented in table IV are obtained. These figures show that in November the average dry weight

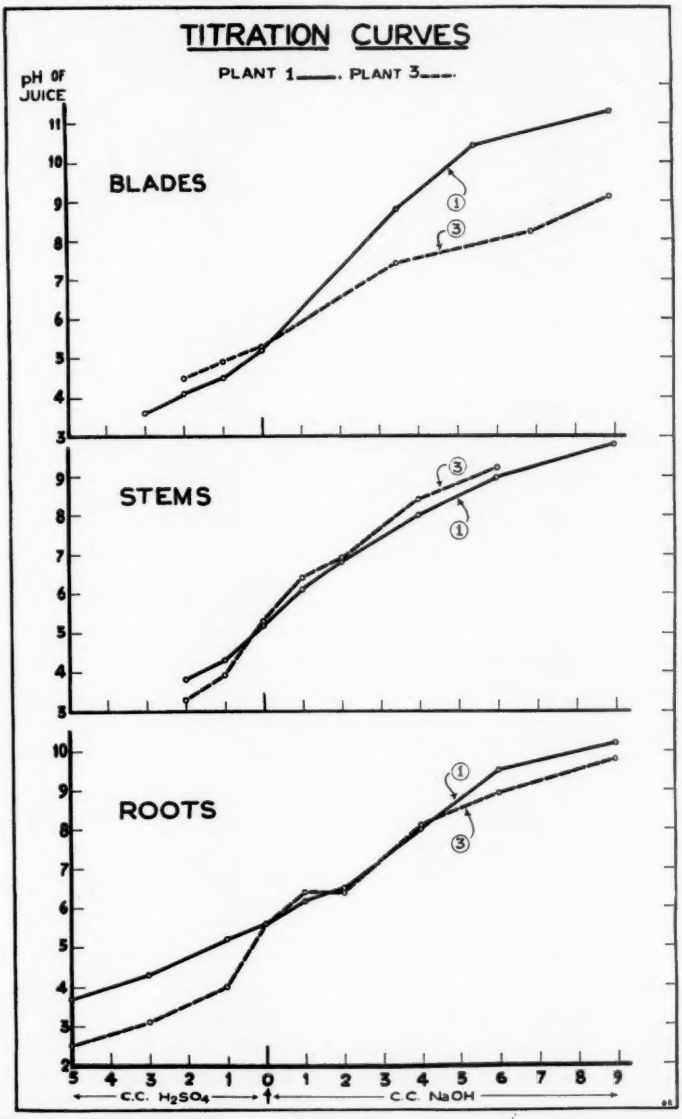


FIG. 1. Effect of addition of varying amounts of acid and alkali upon hydrogen ion concentration of juice expressed from plants 1 and 3.

TABLE II

NITROGEN ANALYSES OF PLANTS HARVESTED NOVEMBER 20, 1931, NINE WEEKS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	AMINO N	PROTEIN N	NITRATE N	TOTAL N
	%	%	%	%
Blades				
1	0.070 ± 0.011	1.585 ± 0.0004	None	1.655 ± 0.001
2	0.086 ± 0.004	1.605 ± 0.003	None	1.691 ± 0.008
3	0.231 ± 0.001	1.461 ± 0.001	Trace	1.692 ± 0.0004
Stems				
1	0.395 ± 0.006	0.986 ± 0.007	Trace	1.381 ± 0.0007
2	0.463	1.122	Trace	1.585
3	0.441	0.911	None	1.352

TABLE III

NITROGEN ANALYSES OF PLANTS HARVESTED APRIL 27, 1932, 7½ MONTHS AFTER STARTING THE PLANTS IN THE NUTRIENT SOLUTIONS
PERCENTAGES EXPRESSED ON MOISTURE-FREE BASIS

SERIES	AMINO N	PROTEIN N	TOTAL N
	%	%	%
Blades			
1	0.130 ± 0.0009	1.707 ± 0.008	1.837 ± 0.009
2	0.099 ± 0.001	1.573 ± 0.022	1.673 ± 0.020
3	0.148 ± 0.002	1.923 ± 0.008	2.071 ± 0.005
4	0.231 ± 0.007	2.285 ± 0.026	2.516 ± 0.018
5	0.161 ± 0.001	2.139 ± 0.023	2.300 ± 0.024
Stems			
1	0.666 ± 0.019	1.334 ± 0.015	2.000 ± 0.001
2	0.633 ± 0.013	1.294 ± 0.008	1.927 ± 0.022
3	0.344 ± 0.007	1.114 ± 0.002	1.459 ± 0.010
4	0.199 ± 0.002	1.205 ± 0.011	1.404 ± 0.013
5	0.155 ± 0.0007	0.866 ± 0.016	1.021 ± 0.016

of the total blades of series 3 was 36.3 gm., and of series 1, 66.4 gm. At that time the blades of series 1 contained 0.0465 gm. of amino nitrogen per plant, while those of series 3 contained 0.0838 gm. Although weighing only half as much as those of series 1, the blades of series 3 contained almost double the amount of amino nitrogen. This seems to be evidence of a derangement in the synthesis of proteins by the plants deficient in potassium.

TABLE IV

TOTAL AMOUNTS OF PROTEINS WITHIN THE PLANTS

SERIES	AVERAGE DRY WEIGHT OF PLANT		TOTAL PROTEIN N PER PLANT		TOTAL AMINO N PER PLANT	
	NOV.	APRIL	NOV.	APRIL	NOV.	APRIL
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Blades						
1	66.4	189.7	1.052	3.238	0.0465	0.2466
3	36.3	69.7	0.530	1.338	0.0838	0.1032
Stems						
1	13.5	299.4	0.133	3.994	0.0533	2.660
3	4.6	36.0	0.030	0.401	0.0203	0.124

The percentages of sugars in the plants are given in tables V and VI. Table V shows that two months after starting the plants in the nutrient solutions there was no great difference in the percentages of sugars in the three groups of plants studied. A slightly higher percentage of reducing sugars and lower percentage of sucrose is suggested in the blades of the potassium-deficient plants at that time. Although these differences may be within the limits of experimental error, it is possible that they suggest a tendency when compared with the results obtained later. In April the

TABLE V

SUGAR PERCENTAGES OF PLANTS HARVESTED NOVEMBER 20, 1931, EXPRESSED ON MOISTURE-FREE BASIS

SERIES	REDUCING SUGARS	SUCROSE	TOTAL SUGARS
	%	%	%
Blades			
1	3.230 ± 0.094	3.717 ± 0.224	6.947 ± 0.318
2	4.235 ± 0.099	2.487	6.722
3	3.548 ± 0.066	3.183 ± 0.074	6.731 ± 0.005
Stems			
1	4.879 ± 0.079	14.630 ± 0.465	19.509 ± 0.385
2	7.003 ± 0.124	14.163 ± 0.138	21.166 ± 0.013
3	4.155 ± 0.109	13.698 ± 0.011	17.853 ± 0.120

blades of the plants starved for potassium were higher in reducing sugars and lower in sucrose than those of the controls, although the percentages of total sugars remained about the same. The stems of series 2 were higher in reducing sugars and lower in sucrose than the controls, but the stems of

the other potassium-deficient plants were very low in reducing sugars as well as sucrose and there was a positive correlation between the amount of potassium supplied and the total sugar produced. These results indicate derangements in the transformations between the hexoses and sucrose. These derangements were found to be correlated with a weak activity of invertase, which will be discussed later.

3. Invertase studies

In a former paper (**24**) evidence was presented indicating that a deficiency in potassium resulted in a weakened activity of invertase in sugar cane. This has been substantiated and studied in greater detail in the present investigation and a preliminary report of some of the results has already appeared (**25**).

TABLE VI

Sugar percentages of plants harvested April 27, 1932, expressed on moisture-free basis

Series	Reducing sugars	Sucrose	Total sugars
	%	%	%
Blades			
1	4.580 ± 0.050	4.225 ± 0.145	8.810 ± 0.095
2	4.360 ± 0.028	5.622 ± 0.014	9.982 ± 0.014
3	5.617 ± 0.134	2.559 ± 0.212	8.176 ± 0.078
4	5.973 ± 0.188	3.070 ± 0.044	9.044 ± 0.145
5	5.346 ± 0.087	3.647 ± 0.005	8.993 ± 0.081
Stems			
1	8.538 ± 0.045	26.220 ± 0.112	34.758 ± 0.157
2	10.055 ± 0.231	21.431 ± 0.053	31.487 ± 0.117
3	8.024 ± 0.057	23.116 ± 0.257	31.140 ± 0.314
4	3.483 ± 0.049	23.747 ± 0.472	27.230 ± 0.522
5	3.653 ± 0.160	18.980 ± 0.160	22.634 ± 0.000

Determinations were made of the activity of the invertase of the blades and the stems of the material harvested in November and in April, both unbuffered and at the optimum reaction. The buffers used in these experiments were those of McIlvaine which are described by Clark (**8**). These were chosen because they do not contain potassium. Controls were run on the hydrogen ion concentration of the tests before and after inversion, which showed that the buffers were efficacious. It was also found that the reaction of the unbuffered tests did not change materially during inversion.

Determinations were made of the effect of the hydrogen ion concentration upon the activity of invertase of the plants of series 1, the results of which are given in table VII.

The results of all the invertase tests are reported as cc. N/20 $KMnO_4$, the Bertrand titration method having been used. With the exception of the effect of the reaction on the invertase activity of the blades harvested in

TABLE VII

pH AND INVERTASE OF SERIES 1

pH	Inversion by H+	Inversion by invertase			
		November		April	
		Blades	Stems	Blades	Stems
		cc.	cc.	cc.	cc.
3.3	7.77	.....	.....	23.30	.....
3.7	4.71	17.9	52.30	29.91	21.07
4.0	1.83	18.9	59.87	34.09	23.21
4.4	0.90	18.6	62.11	38.27	25.54
4.9	1.39	18.25	49.85	28.87	17.47
5.3	1.15	17.5	.....	20.31	.....
5.9	.....	16.9	.....	.....	.....

November, all the tests were conducted in duplicate, with duplicate controls for each lot of material and for each buffer, and were repeated. Table VII shows that the optimum reaction for the invertase of the controls was about pH 4.4.

Invertase determinations of all the plants are presented in table VIII. The determinations of the activity in the blades and stems, buffered and unbuffered, were performed on separate days. While every effort was made to keep the conditions uniform, identical conditions were impossible of attainment. Consequently the results in table VIII are only roughly comparable vertically. They are intended to be read horizontally.

Table VIII shows that the blades and stems of the plants supplied with potassium had a greater invertase activity than those of the potassium-deficient plants. The difference was greater in the stems than in the blades. Greater differences would have been obtained in the stems of the April material if a distinction had been made between green and dry leaf cane, a point which will be discussed later. When tested at the optimum reaction, the activity was apparently equal in all the blades. The activity of the stems was not equalized at the optimum reaction, however, although the difference between the extremes was less than when tested unbuffered.

Because of the importance of hydrogen ion concentration in invertase activity, which is illustrated in table VII, one might suppose that the dif-

TABLE VIII

INVERTASE ACTIVITY IN SUGAR CANE

TEST	SERIES						
	1	2	3	4	5	6	7
	November material						
Blades (unbuffered)	42.00	.....	39.10	28.25	33.89		
Blades pH 4	21.9	21.3	20.16	22.5	22.5		
Stems (unbuffered)	56.45	33.10	16.68	18.65	19.97		
Stems pH 4.4	50.70	42.04	27.42	26.67	22.62		
	April material						
Blades (unbuffered)	25.00	21.21	22.64	17.11	17.71	.....	.....
Blades pH 4.4	36.08	36.16	40.64	39.43	36.69	35.19	34.77
Stems (unbuffered)	22.16	15.64	24.10	10.52	7.97	.....	.....
Stems pH 4.4	23.04	14.82	19.78	15.78	13.57	.....	.....

ferences in activity shown in table VIII were caused by differences in reaction of the unbuffered plant material. This is not true. Colorimetric determinations of the hydrogen ion concentration of the blade and stem powder were made and no correlation was found between the reaction of the powder and the activity of invertase, as will be seen in table IX. For example, in the April material the blades of series 4 and 5 had equal invertase activity when tested unbuffered but differed in reaction, while series 3 and 4 had the same reaction but differed in activity. In the stems, series 1 had the least favorable reaction but that material was one of the strongest in invertase activity. It is evident that hydrogen ion concentration is not the only factor governing the activity of invertase.

Another factor affecting the activity of invertase in sugar cane is the amount of potassium present in the tissue. The stem powder of the plants of series 1 contained 1.504 per cent. potassium, while that of series 5 had only 0.278 per cent. potassium. An attempt was made in the study reported in 1929 (**24**) to equalize the potassium in the two lots of material; the result was an equal increase in activity in both the control and

TABLE IX

pH OF AIR-DRY POWDER

SERIES	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
	pH	*pH*	*pH*
1	5.6	5.2	5.7
2	5.6	5.2	4.6
3	5.6	5.4	4.6
4	5.8	5.4	4.8
5	5.8	6.0	4.9
6	.....	5.3	5.2
7	.....	5.4	4.9

the potassium-deficient powder. It was concluded at that time that potassium is essential for the formation of invertase within the plant. Unfortunately no buffered tests were made. In the present study the activity of the stems of series 1 and 5 was determined at pH 4.4, with and without the equalization of potassium. The results are reported in table X. The potassium content of the stem powder of these plants was approximately equalized by the addition of potassium dihydrogen phosphate to series 5 and sodium dihydrogen phosphate to series 1. It will be seen that the addition of sodium phosphate to series 1 had no effect, while the potassium phosphate increased the invertase activity of series 5. These data indicate that potassium is a specific accelerator for the activity of invertase in sugar cane, and that sodium and phosphorus are not.

TABLE X

POTASSIUM AND INVERTASE OF STEMS AT PH 4.4

SERIES	POTASSIUM	ACTIVITY ALONE	ACTIVITY WITH EQUAL POTASSIUM
	%		
1	1.504	17.44	18.07 (+ Na)
5	0.278	10.85	13.88 (+ K)

When all the tests for stems are averaged it is found that when tested unbuffered, series 1 is 2.78 times as active as series 5; at the optimum reaction, series 1 is only 1.71 times as active as series 5; and when the potassium is equalized at the optimum reaction, series 1 is only 1.31 times as active as series 5. Evidently the invertase is present within the potassium-deficient plants, but it needs the proper conditions for its optimum activity.

The plants deficient in potassium showed derangements in the transformations of the sugars correlated with the weak activity of invertase. This

should be expected since invertase is the enzyme which catalyzes the hydrolysis and synthesis of sucrose. Direct evidence for the latter has recently been obtained by OPARIN and KURSSANOW (39). The idea occurred that other factors which curtail the production of sucrose from the hexoses within the plant might operate by their effect upon the activity of invertase.

To obtain additional evidence on this point a supplementary experiment was performed. At the Waipio Substation of the Hawaiian Sugar Planters' Association there was a field of variety P.O.J. 2878 which had received uniform fertilizer and irrigation treatment. For some undetermined reason the juices in the cane in one portion of the field were consistently low in sucrose, while in another part of the same field juices high in sucrose were obtained. The object of the experiment was to determine the juice quality and invertase activity of the plants giving good and poor juices. Five plants of each lot were taken and were subdivided into blades, green leaf cane, and dry leaf cane. The quality of the juices is given in table XI, these data being supplied by the Department of Sugar Technology of this Station.

The invertase activity of these samples is given in table XII. The term green leaf cane is applied to that portion of the millable cane to which green leaves are attached, while dry leaf cane is the part which bears dry leaves or none.

TABLE XI

JUICE QUALITY OF CANE

SAMPLE	BRIX*	POLARIZATION*	PURITY*	QUALITY RATIO*
Good cane (green leaf part)	13.60	9.60	70.6	16.41
Poor cane (green leaf part)	12.89	7.23	56.1	28.73
Good cane (dry leaf part)	22.12	20.30	91.8	6.39
Poor cane (dry leaf part)	20.87	18.84	90.3	6.95

* Purity is the ratio between the polarization (or total sucrose) and the Brix (or total solids measured by the Brix hydrometer), while quality ratio is the number of tons of cane necessary to make one ton of sugar.

It will be seen that when tested unbuffered, the activity of the stems of the cane giving good juices is the same as that of the poor cane. The difference between the activities of the blades of the two lots of plants is not great, but may be significant. The most striking point brought out by this

TABLE XII

INVERTASE ACTIVITY, VARIETY P.O.J. 2878

SAMPLE	CANE GIVING GOOD JUICES	CANE GIVING POOR JUICES
Blades	12.20	9.37
Green leaf cane	12.71	13.99
Dry leaf cane	6.07	6.88

experiment is the fact that the activity in the green leaf part of the stem is double that of the dry leaf portion. This applies to both the good cane and the poor. In the good cane the invertase of the blades is equal in activity to that of the green leaf portion of the stalk. It is evident that wherever sucrose is actively made or stored, invertase activity is great; whereas in the dry leaf portion of the stick, where the storage of sucrose is practically completed, the invertase activity decreases.

4. AMYLASE STUDIES

In the study reported in 1929 (**24**) the activity of amylase was found to be greater in the plants deficient in potassium than in the controls, in both blades and stems. It was suggested that the cause of the increased activity in the plants deficient in potassium might be their greater percentage of sugars, which perhaps constitute the substrate necessary for the formation of starch by amylase, since the amount of substrate has been found in certain cases to affect the amount of enzyme produced. Another possibility suggested was an increase in the absorption of phosphorus in the potassium-deficient plants, since phosphorus is known to favor the activity of amylase, and JOHNSTON and HOAGLAND (**31**) found that tomato plants absorbed an increased amount of phosphorus when the supply of potassium was deficient.

In the present studies, determinations were made of the optimum reaction for the amylase of cane; of the activity in the blades and the stems of the plants supplied with varying amounts of potassium; and of the effects of potassium, phosphorus, calcium, magnesium, dialysis, and sugars upon the activity of amylase. The results are given as color with iodine-potassium iodide after incubation for 20 hours at 36.5° C., using soluble starch as the medium. The following twelve colors are used to represent the course of the digestion of starch, in the order named: blue, purple-blue, purple, red-purple, very dark red, dark red, red, bright red, orange-red, orange, pink-yellow, yellow.

TABLE XIII

pH AND AMYLASE OF SERIES 1

pH	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
2.2	Purple-blue	.....	.....
4.9	Dark red	Dark red	Red-purple
5.3	.....	Dark red	Red
5.9	Bright red	Bright red	Red
6.3	.....	Red	Red
7.1	.....	Red-purple	Red
8.1	Red-purple	.....	.....

The effect of the reaction of the medium upon the amylase activity is shown in table XIII. The optimum reaction for the amylase of blades was found to be pH 5.9. The amylase of stems did not seem to be influenced appreciably by hydrogen ion concentration.

The amylase activity of all the plants is given in table XIV.

TABLE XIV

AMYLASE OF PLANTS GROWN WITH VARYING AMOUNTS OF POTASSIUM

SERIES	NOVEMBER MATERIAL		APRIL MATERIAL	
	BLADES (pH 5.9)	STEMS (UNBUFFERED)	BLADES (pH 5.9)	STEMS (UNBUFFERED)
1	Very dark red	Orange-red	Red-purple	Red-purple
2	Dark red	.....	Red	Purple
3	Orange	Yellow	Orange	Red
4	Yellow	Yellow	Yellow	Orange
5	Pink-yellow	Yellow	Orange-red	Bright red
6	.....	.....	Dark red	Dark red
7	.....	.....	Dark red	Dark red

The amylase activity of the blades of the November material in decreasing order was $4 > 5 > 3 > 2 > 1$. That of the blades of the April material was $4 > 3 > 5 > 2 > 6$ and $7 > 1$. That of the stems collected in April was $4 > 5 > 3 > 6$ and $7 > 1 > 2$. In every test the plants deficient in potassium had greater amylase activity than the controls.

Studies were made concerning the cause of the active amylase of the potassium-deficient plants:

a. Sugars

Perhaps the formation of starch from dextrose in plants is catalyzed by amylase. If so, and if the production of amylase is regulated by the amount of dextrose, then the plants which have the most active amylase should also contain the largest percentage of reducing sugars, other things being equal. The data for the sugars in the November harvest are insufficient for this comparison. Table VI shows that the percentage of reducing sugars in the blades of the April material decreased in the order $4 > 3 > 5 > 2 > 1$. The amylase of the same blades differed in the same order. The stems of the plants deficient in potassium, however, had the lowest percentage of reducing sugars and the most active amylase. In short, although the principle of the quantitative regulation of enzyme production might apply to the amylase activity in the blades, it could not be applied in the stems.

Before the sugar determinations were performed a supplementary test was conducted to determine the effect of sugars upon the activity of amylase. Cane shoots were taken from cuttings and supported in split corks in Erlenmeyer flasks, and were placed in intense diffused light. One plant was given distilled water, another a 1 per cent. solution of sucrose, and a third a 1 per cent. solution of dextrose. The sugars and water had been boiled and cooled. They were renewed at intervals. After four days the blades were removed, ground and dried, and amylase determinations were performed. At the time of the removal of the blades the three plants appeared about the same, except that the spindle of the plant which had received dextrose was greener than the spindles of the other two plants. It was found that the plant which had received dextrose had the most active amylase, that which had been in water was the least active, while the amylase of the plant receiving sucrose was intermediate in activity. These results are not in agreement with those of SJÖBERG (**43**), who found that bean plants receiving sugars had weaker amylase activity in stems and leaves than those with no organic nutrients. The present results seem to uphold the general theory of the effect of the substrate upon the production of enzymes, although, as explained above, that theory cannot explain the amylase activity in the stems in these potash investigations.

b. Phosphorus

The percentages of phosphorus in the plants were given in tables XV and XVI of the first paper of this series (**26**). The relative amounts of phosphorus, potassium, and amylase are given in table XV, in which the

TABLE XV

RELATIVE AMOUNTS OF PHOSPHORUS AND POTASSIUM, AND AMYLASE ACTIVITY

	NOVEMBER BLADES	APRIL BLADES	APRIL STEMS
Phosphorus	5 > 4 > 3 > 2 > 1	4 > 5 > 3 > 2 > 1	5 > 4 > 1 and 3 > 2
Potassium	1 > 2 > 3 > 5 > 4	1 > 2 > 3 > 5 > 4	1 > 2 > 3 > 4 > 5
Amylase	4 > 5 > 3 > 2 > 1	4 > 3 > 5 > 2 > 1	4 > 5 > 3 > 1 > 2

figures are the series numbers of the plants. From this it will be seen that there is a fairly good positive relationship between the phosphorus content and the amylase activity. There is also a good negative correlation between the potassium content and the amylase activity.

Tests were conducted with the blades of the material collected in both November and April to determine whether equalizing the phosphorus would result in the equality of the activity of amylase and it was found that it did not. Evidence was obtained, however, indicating that the amylase of the potassium-deficient blades is activated by phosphorus more than is the amylase of the control blades, as shown in table XVI.

TABLE XVI

AMYLASE ACTIVATION BY PHOSPHORUS

SERIES	TREATMENT	COLOR WITH IKI
Blades		
1	H_2O	Dark red
1	Na phosphate	Dark red
2	H_2O	Orange-red
2	Na phosphate	Orange

c. Theories more or less disproved

Aspargine is known to be an activator of amylase. A microchemical test was made of the blades of series 1 and 4, using copper acetate. No marine blue crystals of copper asparginate were found, indicating that little or no asparagine was present.

Sodium is one of the ions known to activate amylase, but the plants of series 5 were grown entirely without sodium and their amylase was about equal in activity to the amylase of series 4.

It might be supposed that the potassium content of the control plants was high enough to exert a direct inhibitory action upon amylase. This

was not true because the addition of potassium to the blades of the control plants harvested in November resulted in a slight increase in activity.

OPARIN and DJATSCHKOW (**38**) concluded from studies with wheat that amylase is made in the plant and goes to the grain. Amylase might be made in the blades of the sugar cane plant, its production being controlled by the sugar content of the blades rather than of the stems. But if amylase migrates from the blades to the stems it would supposedly go through the phloem. The phloem necrosis which curtailed the translocation of proteins and sugars from the blades to the stems would also prevent the migration, of amylase. This should tend to result in less amylase in the stems of the potassium-deficient plants than in the controls, which was not found.

d. Activation by salt constituents in general

The blades of the potassium-deficient plants harvested in November were higher in total ash, calcium, and magnesium than were the controls, as shown in the first paper of this series (**26**). These differences held for magnesium in the blades and stems of the April material, and for calcium in the blades, but did not hold for calcium in the other organs or for total ash. Therefore, although the higher ash, calcium, and magnesium content of the potassium-deficient plants might explain their more active amylase in the material of the November harvest, only the magnesium content could apply to that collected in April.

The magnesium contents of the blades of series 1 and 2, harvested in November, were equalized by the addition of magnesium sulphate to the former. It was found that the magnesium sulphate activated the amylase of the blades of series 1 slightly but did not make it equal to that of series 2. Similar results were found when the calcium content was equalized.

To find the effect of the ash constituents in general, the blades of the November harvest were dialyzed in collodion bags before incubation. Pre-

TABLE XVII

AMYLASE ACTIVITY OF BLADES AFTER DIALYSIS FOR 24 HOURS

SERIES	COLOR WITH IKI	
	NON-DIALYZED	DIALYZED
1	Dark red	Red-purple
2	Red	Dark red
3	Pink-yellow	Orange
4	Yellow	Pink-yellow
5	Yellow	Pink-yellow

liminary tests and controls showed that the amylase of sugar cane is non-dialyzable through collodion. The amylase activity of the blades with and without previous dialysis for 24 hours is shown in table XVII.

In every plant, dialysis for 24 hours resulted in decreased activity, indicating the loss of an activator. In another experiment dialysis was continued for three days. Amylase determinations were then performed, the results of which are given in table XVIII.

Dialysis for three days thus decreased the amylase activity of series 2, 4, and 5 but increased that of series 1 and 3. This shows that dialysis

TABLE XVIII

AMYLASE ACTIVITY OF BLADES AFTER DIALYSIS FOR 3 DAYS

SERIES	COLOR WITH IKI	
	NON-DIALYZED	DIALYZED
1	Purple	Red-purple
2	Red-purple	Purple
3	Very dark red	Dark red
4	Pink-yellow	Red
5	Pink-yellow	Dark red

even for three days does not equalize the activity of amylase. It is therefore not the mere presence of the ash constituents which alone determines the activity of amylase. It should not be surprising that dialysis at times results in increased activity of amylase and at other times decreases its action. Among the dialyzable constituents of the plant material it seems only reasonable that there are some which increase and some which decrease the activity of amylase, and that these diffuse at different rates.

Further considerations of the amylase activity will be found in the discussion.

Other tests to be reported in another contribution have shown greater dextrinase and weaker maltase activity in the blades of cane plants deficient in potassium than in the controls. Greater differences in maltase activity were found in wheat and buckwheat than in sugar cane.

5. EREPTASE STUDIES

The optimum reaction for the activity of ereptase was determined and the results are shown in table XIX. The test consisted of the formation of tryptophane from Witte peptone, the amount of tryptophane being de-

termined by the addition of bromine water. This gives a pink color and the deeper the pink the greater the amount of tryptophane, and hence the greater the activity of ereptase.

TABLE XIX

PH AND EREPTASE OF SERIES 1

pH	November material		April material		
	Blades	Roots	Blades	Stems	Roots
2.2	Colorless*	.....	.....	.....	.....
3.3	.....	Colorless	Lightest pink	.....	.....
3.7	.....	Colorless	Lighter pink	.....	Colorless
4.0	.....	Light pink	Light pink	Colorless	Very light pink
4.4	.....	.....	Pink	Colorless	Light pink
4.9	Light pink	Very light pink	Rose	Colorless	Pink
5.3	.....	.....	Light pink	Light pink	Light pink
5.5	.....	.....	.....	Pink	.....
5.9	Colorless	.....	.....	Deeper pink	Colorless
7.0	.....	.....	.....	Light pink	.....
8.1	Colorless	.....	.....	Colorless	.....

* Negative results; blanks indicate no test performed.

The optimum reaction for the blades, November and April, was found to be pH 4.9, stems 5.9, and roots about pH 4.9.

Determinations were made of the activity of ereptase of the blades and roots of the plants harvested in November, and of the blades, stems, and roots of the April material, all the tests being made at the optimum reaction. No difference was found in the activity in the blades. Some slight differences occurred in the stems, but they were not correlated with the amount of potassium supplied nor with any other observed factor. The activity in the roots of the material harvested in April occurred in the order $4 > 3$ and $5 > 2$ and $7 > 1 > 6$. The gradation is not perfect, but it does indicate greater activity in the plants deficient in potassium.

6. Peptase studies

Peptase activity was found in the studies reported in 1929 (**24**) to be greater in the controls than in the plants deficient in potassium. Since

peptase may be important in catalyzing the synthesis of proteins, any derangement in its activity might be expected to reduce the production of protoplasm and hence curtail growth.

It was hoped to repeat the studies of peptase in the present investigations, using a more exact method. To date no evidence of peptase activity has been obtained. Using the carmine fibrin method of GRÜTZNER (**22**), no peptase activity was detected in blades or roots at pH 2.2–5.9. With the titration of WILLSTÄTTER and WALDSCHMIDT-LEITZ (**55**) no proteolytic action was demonstrated at pH 2.2, 3.3, 4, 4.9, 7.1, or 8.1. In these tests water was used as the medium for extraction. According to VINES (**51**) ereptase is generally more readily extracted by water while peptase is extracted more easily by 2 per cent. NaCl. However, where plants have been grown in a concentrated nutrient solution, VINES found that peptase could be extracted with water. Inasmuch as the nutrient solutions used in the Chicago experiments were much more concentrated than those used in the present studies, the idea occurred that the peptase of the former plants might have been more readily soluble in water than that of the present investigation, which might require additional salt for extraction. To determine this point, blade material was extracted for two days in 2 per cent. NaCl. The reaction was then adjusted to pH 5.2–5.3 with sodium dihydrogen phosphate, and a peptase test was performed using carmine fibrin. No sign of digestion occurred. The WILLSTÄTTER-WALDSCHMIDT-LEITZ method was employed at pH 4.9 and 5.4, with negative results.

The conclusion is drawn that under the conditions of the present investigations, no peptase was found. Possibly using a different method of extraction or a different hydrogen ion concentration, positive results would be obtained. Perhaps the more concentrated nutrient solutions used in the former study (**24**) caused the development of peptase strong enough to be detected. Peptase is probably present in cane but in such small amounts that it is often undetectable. For the study of the relation of potassium to the activity of peptase, soy beans or other plants high in proteins might be more desirable.

Notwithstanding the fact that no evidence of peptase was obtained, derangements in the formation of proteins were indicated. Growth was proportional to the amount of potassium supplied. In November the dry weight of the blades of series 3 was about half that of series 1, whereas series 3 contained nearly double the amount of amino nitrogen, as shown in table IV. This would seem to indicate difficulty in some step of the synthesis of proteins. Because the activity of ereptase was equal in all the blades, the indications are that the synthesis of the higher proteins was curtailed by the deficiency of potassium. Whether or not this was due to a weak activity of peptase as shown previously (**24**) could not be ascer-

tained in the present study. The analogy between the nitrogen and sugar data is interesting although inconclusive.

Discussion

Since the chemical transformations in plants are chiefly catalyzed by enzymes, it would seem that quantitative determinations of their activity in connection with chemical analyses in studies of mineral deficiencies might lead to important results. A few studies of the effects of potassium upon the activity of enzymes will be mentioned. DOBY and HIBBARD (**10, 11**) found more active invertase and diastase in sugar beets deficient in potassium than in those supplied with that element. HARTT (**24**) reported derangements in the activities of invertase, diastase, peptase, and catalase in sugar cane plants grown without potassium. ECKERSON (**12**) found that plants low in potassium are weak in reducase activity. In the present studies a decreased activity of invertase and increased activity of amylase were found in the cane plants deficient in potassium. It would seem that these derangements in enzyme action might be closely connected with the disturbance in the protein and carbohydrate metabolism of the plant.

While it is probably not essential to consider that all of the reactions catalyzed by enzymes are reversible, yet this has been proved for several and assumed for many. The supposition that there is a separate enzyme which condenses dextrose and levulose to form sucrose does not seem necessary in view of the recent work of OPARIN and KURSSANOW (**39**), who have demonstrated the synthetic action of invertase. Their report provides a summary of the subject of enzymatic synthesis. In the studies, the views that sucrose is not the first sugar formed in photosynthesis, and that both the synthetic and the analytic reactions are catalyzed by the same enzymes, are taken as working hypotheses. It is realized that further studies may disprove these points, but at present they seem to explain the results obtained.

The enzymes of sugar cane have been insufficiently studied. BROWNE (**4**) mentioned the presence of the following enzymes in cane: diastase, invertase, oxidase, and a reducing enzyme. In addition to these, BROWNE and BLOUIN (**5**) stated that peptonizing enzymes are present in cane stalks. HARTT (**24**) reported the results of quantitative determinations of diastase invertase, peptase, ereptase, and catalase. NEEB (**36**) reported the occurrence of saccharase, amylase, catalase, tryosinase, oxidase, peroxidase, and maltase.

PROTEIN METABOLISM

The subject of the utilization of potassium by plants, with particular reference to the interrelationships between carbohydrate and protein

metabolism in plants deficient in potassium, has been so well summarized by NIGHTINGALE *et al.* (**37**) in connection with studies of the tomato plant that it does not need to be repeated here. In substance, they conclude that where carbohydrates accumulate in potassium-deficient plants, a principal cause of their accumulation is a derangement in the synthesis of proteins. A similar conclusion had previously been reached by HARTT (**24**), who found higher percentages of total sugars and increased formation of lignin correlated with a deficiency in the activity of peptase in sugar cane plants grown without potassium.

In the present studies, derangements in the synthesis of proteins in the plants deficient in potassium were indicated indirectly by the curtailment of growth (**26**) and directly by the nitrogen determinations. Two months after starting the plants in the nutrient solutions, the blades of series 3 had a higher percentage of amino nitrogen and a lower percentage of protein nitrogen than those of series 1, the total nitrogen remaining about the same. Similar though less pronounced differences were found in the stems, as shown in table II. Not only was there a difference in percentages, but the actual amount of amino nitrogen in the blades of series 3 was almost double that in series 1, although the average dry weight of the plants of series 1 was about twice that of those of series 3, as shown in table IV. This accumulation of amino nitrogen in the blades and stems of the plants deficient in potash which occurred at the age of two months, together with the slight decrease in percentage of protein nitrogen, seems to be evidence of the failure of the potassium-deficient plants to synthesize proteins as usual. Attention is called to the fact that the curtailment in synthesis occurred after the formation of amino acids rather than before, indicating that in these plants the reduction of nitrates proceeded as usual. Notwithstanding this point, reducase determinations would be interesting and would have been made had time permitted.

The terminology of proteolytic enzymes has become considerably complicated in recent years. Following VINES (**51**), in this paper the simple distinction between peptase and ereptase is made, the former catalyzing the digestion of complex proteins to peptones and allied compounds, and the latter carrying the digestion from these intermediate products to amino acids. Since there is little evidence of a tryptic enzyme occurring generally in plants, that is left out of consideration. In the studies reported in 1929 (**24**) both peptase and ereptase were found to occur in cane, whereas in the present study ereptase was the only proteolytic enzyme detected. As mentioned in the results, the use of other solvents at other reactions might have shown the presence of peptase. Inasmuch as the activity of ereptase seemed not to be affected by the supply of potassium, with the possible exception of the roots, derangements in ereptase cannot explain the deficiency

in the synthesis of proteins. If the synthesis of proteins follows the same steps as their digestion, would not a weakened peptase activity result in the accumulation of peptase and proteoses rather than amino acids? It is, however, conceivable that both might occur. It is unfortunate that no determinations of the forms of nitrogen were made in the Chicago studies, and that the attempted detection of peptase in the Honolulu studies failed. Since at the age of two months the synthesis of proteins in the potassium-deficient plants had proceeded as usual, as far as the production of amino nitrogen, it seems likely that either some essential amino acids were lacking or that a derangement occurred in the activity of the higher proteolytic enzymes. There is need for further work along these lines.

Although in November the stems of the plants deprived of potash were slightly higher in amino nitrogen and lower in protein nitrogen than were the controls, in April the stems of the potassium-deficient plants had considerably lower percentages of amino, protein, and total nitrogen than the controls. The blades of the plants harvested in April showed higher percentages of amino, protein, and total nitrogen in the plants deficient in potassium than in those supplied with an adequate amount of that element. Thus the proteins of the blades and stems of the plants harvested in November varied in the same way, while in the April harvest the results of the protein analyses of the blades and stems were diametrically opposed. These observations may be explained by assuming that the chief seat of synthesis of amino acids and simple proteins is in the blades; that at least some water and nutrient salts can still move up in the xylem of the potassium-deficient plants; but that a necrosis of the phloem curtailed the translocation of nitrogenous compounds from the blades to the stems. Evidence of phloem necrosis possibly similar to that reported by JOHNSTON and DORE (**30**) has been presented in the first paper of this series (**26**), the evidence consisting of brown discolorations of the sieve tubes and companion cells in the midribs and stems of the plants deficient in potassium. The necrosis might be due either to a disturbance in the synthesis of proteins resulting in abnormal protoplasm in the sieve tubes and companion cells, or to the accumulations of iron at the nodes resulting in the coagulation of the proteins of the phloem, or to both conditions. Whatever the cause, it is apparent that such a necrosis might seriously interfere with the translocation of nitrogenous and carbohydrate compounds.

In short, the results here reported indicate that both the synthesis and the translocation of proteins in the sugar cane plant are decreased by a deficiency in potassium. The derangement in the synthesis of proteins is possibly caused by a weak activity of peptase, as shown by former studies (**24**). The interference with their translocation may be caused by the necrosis of the phloem.

Differences so slight as to be within the limits of experimental error in the November harvest became intensified by the time of the April harvest, when the blades of the plants deficient in potassium had greater percentages of reducing sugars and lower percentages of sucrose than the controls, as shown in table VI. Inasmuch as the activity of invertase was greater in the plants receiving potassium than in those deprived of that element, it would seem that invertase aids in the synthesis of sucrose in the blades of sugar cane.

In the stems of the plants harvested in April a distinction must be made between partial and complete starvation for potassium. The plants of series 2, which were only partially starved for potash, reacted the same way as the blades, having a higher percentage of reducing sugars and lower percentage of sucrose than the plants of series 1. The other plants were more severely affected, the percentages of reducing sugars, sucrose, and total sugars all being lower in series 3, 4, and 5 than in series 1. The activity of invertase was weaker in the plants starved for potash. The supply of dextrose, the essential source of energy for growth and life, was curtailed especially in the stems of series 4 and 5, both by the necrosis of the phloem which would interfere with the translocation of sugars from the blades to the stems, and by the weak activity of invertase which would decrease the inversion of the sucrose already in the stems.

The suggestion is therefore made that a deficiency in potassium in the sugar cane plant may interfere with the transformations between the hexoses and sucrose, both anabolic and catabolic, as well as with their translocation.

From the practical standpoint the object of the sugar planter is to obtain the plant which stores the most sucrose in the stems. Under field conditions it is unlikely that any plants as deficient in potassium as the plants of series 3, 4, and 5 would continue until harvest. Their weakened condition is so obvious that further applications of fertilizer would be made, or else they would probably die. The important distinction for the planter to make is between plants such as series 1 and 2, those plants which appear very much the same in color, size, and general condition but which show a difference in the amount of sucrose in the stems. The stems of series 2 were higher in reducing sugars and lower in sucrose than those of series 1, and the activity of invertase in series 2 was weaker than that of series 1. If plants in the field similar to the plants of series 2 could be recognized in time and given the proper fertilizer, it seems possible that the activity of their invertase could be increased and so increase the amount of sucrose stored in the stems.

The relationship between potash fertilization and yield is obscure. VAN DEN HONERT (**50**) found a low figure for Brix in variety P.O.J. 2878 deficient in potassium, but suggested that this might be caused by the fact that the suckers of the plants starved for potash were younger and less mature than the controls. As mentioned in the discussion of the first paper in this series (**26**), the results of some experiments have shown an increase in yield of sugar following the application of potash, others have shown no response, and still others have indicated a depressing effect, the last condition possibly resulting from a depression in the absorption of phosphorus. It is evident that a delicate relationship exists. While it is by no means suggested that the only factor involved is the activity of invertase, yet the present results show that enzyme to be important, and it is felt that further attention should be paid to the activity of invertase in determining the effects of fertilizer applications and other cultural practices. This matter is discussed more fully below.

For a complete understanding of the carbohydrate metabolism of potassium-deficient plants, certain fundamental principles should be determined. Is sucrose synthesized in the leaves by the enzyme invertase? Is sucrose merely a temporary storage product in the leaves, converted into hexoses before translocation to the stems, or is sucrose the main translocation form of sugar, or are both the hexoses and sucrose translocated? These questions being of general importance are summarized in textbooks of plant physiology. The investigations with sugar cane have been discussed by VISWANATH (**52**), who concluded that the bulk of evidence seems to favor the view that sucrose is built up in the stem from reducing sugars sent into it by the leaf. GEERLIGS (**19**), however, is of the opinion that sucrose, dextrose, and levulose are all continuously supplied by the leaf.

The writer is now conducting studies on these points and the results will be reported later. In the meantime it would seem that the present studies offer indirect evidence that sucrose in the sugar cane plant is synthesized by invertase, as already mentioned in a preliminary report (**25**). The blades of the plants deficient in potassium had the highest percentage of reducing sugars and lowest percentage of sucrose, and they also had the weakest activity of invertase. Since the percentage of total sugars remained about the same in the blades, it would seem that this is evidence of a derangement in the forms of sugars rather than a difference caused by some other factor, such as photosynthesis or protein formation. Interference with translocation may have affected the results in the material harvested in April, since both sucrose and hexoses were low in the stems of the plants deficient in potash, with the exception of the plants of series 2. If the action of invertase is only hydrolytic, then what is the explanation of the greater percentage of reducing sugars and lower percentage of sucrose in the plants

which had the weakest invertase activity? Further evidence of the importance of invertase in the synthesis of sucrose is found in the supplementary experiment using variety P.O.J. 2878, described earlier in this paper. Millable cane may be divided into two physiologically different regions: the green leaf cane (or that portion which bears green leaves) and the dry leaf cane (the part bearing dry leaves or none). It is in the green leaf portion of the stem that the most active storage of sucrose occurs, although some additional storage takes place in the dry leaf cane. As shown in table XII, the invertase activity of the green leaf cane was found to be double that of the dry leaf cane. The invertase of the blades was found to be equal in activity to that of the green leaf cane. It is evident that wherever sucrose is actively formed or stored, the activity of invertase is strong, whereas where the process of storage is practically completed, invertase decreases in activity.

The question of the accumulation of carbohydrates in plants deficient in potassium may next be considered. This has already been discussed with references to the literature in an earlier paper (**24**) and also by NIGHTINGALE (**37**). In the present study no evidence of carbohydrate accumulation was obtained. In the blades the total sugars were about the same in all five groups, while in the stems of the plants harvested in April there was a positive correlation between the amount of potassium supplied and the percentage of total sugars formed. As pointed out by NIGHTINGALE, the accumulation of carbohydrates may be an early condition in plants deficient in potassium, disappearing later. Inasmuch as the plants of the present study grew so much more rapidly than those of the former investigation, it may be that at the time of the first harvest the period of carbohydrate accumulation had already passed, if any occurred at all.

It has been shown so far that the utilization of potassium by the sugar cane plant involves the synthesis and translocation of both proteins and carbohydrates. Potassium may affect the synthesis of proteins through its effect on the enzyme peptase, as shown previously (**24**). Potassium affects the transformations of the sugars through its effect on invertase. The question of how potassium affects invertase may next be considered.

Various possibilities suggest themselves as to how potassium affects the activity of invertase: (1) the relationship may be direct, through its entering into the chemical composition of the enzyme, or through its effect as a specific activator; (2) it may be indirect, regulating the hydrogen ion concentration, or the buffer system; (3) it may be antagonistic, in offsetting the effects of inhibitory factors. In a previous report (**24**) the importance of potassium in the formation of invertase was stressed; those tests, however, were all unbuffered. Inasmuch as the activity of invertase in all the blades was equalized at the optimum reaction, it is now felt that potassium is not

essential for the formation of invertase. Because of the equalization of activity at the optimum reaction, it might be concluded that the differences in invertase activity in the blades are due solely to differences in hydrogen ion concentration. This is erroneous since, as shown in table IX, the blades of series 4 and 5 had equal invertase activity when tested unbuffered but differed in reaction; while those of series 3 and 4 had the same reaction but differed in activity. Regarding the possibility of there being differences in the buffer systems, this matter was considered, and it will be seen from figure 1 that little if any difference was found to exist. The suggestion that potassium may offset the effects of inhibitory factors, particularly salt constituents, deserves consideration since, as shown by FALES and NELSON (**14**), the addition of sodium chloride had practically no effect on the velocity of the hydrolysis of sucrose by yeast sucrase at the optimum reaction; but at all other reactions the salt inhibited the action of the enzyme. The greater ash content of the potassium-deficient plants harvested in November might inhibit invertase, but this explanation could not apply to the April material, because in that harvest the plants of series 1 and 2 had the greatest ash content.

Thus either there was some undetermined inhibitory factor, or potassium is a specific activator for invertase in sugar cane, in which latter case the addition of potassium to the enzyme tests should result in increasing the invertase activity; and equalizing the potassium in material from potassium-deficient and control plants should result in approximately equalizing the activity of invertase. Such an experiment was conducted, the results of which are given in table X. Enough potassium dihydrogen phosphate was added to the stem material of series 5 to equalize the potassium in series 1 and 5, and sodium dihydrogen phosphate was added to series 1 to maintain such conditions as hydrogen ion and osmotic concentrations. The test was conducted at pH 4.4. It was found that the addition of potassium to series 5 did not make the activity equal to that of series 1, but did increase it somewhat. As already mentioned, when tested unbuffered, the stem material of series 1 was 2.78 times as active as that of series 5; but when potassium was equalized at the optimum reaction, series 1 was only 1.31 times as active as series 5 (averages of all tests). Since the addition of sodium phosphate to series 1 did not increase the activity, while the potassium phosphate did activate the invertase of series 5, it would seem that potassium is a specific accelerator for the enzyme invertase. If this is true for other plants which store sucrose then it may help to explain the statement of COLIN and BILLON (**9**) that potassium plays a direct rôle in the formation of sucrose in beets.

The preceding discussion shows that the invertase of stems is affected more severely by potassium deficiency than is that of blades. The differ-

ence between the extremes is much greater in the stems than in the blades (table VIII), and the activity in the blades is equalized at the optimum reaction while that in the stems is not. Although a possible explanation is the higher percentage of potassium in the blades than in the stems, as shown in table XVI of the first paper in this series (**26**), yet the subject is too complex to permit complete understanding at the present time.

The accelerating effect of potassium may help to explain the differences in invertase activity in the green leaf cane as compared with that in the dry leaf cane. Table XII shows that the activity in the green leaf cane is double that of the dry leaf cane. Analyses performed by the Chemistry Department of this Station have shown that the percentage of potassium increases from the bottom to the top of the stalk. While other factors undoubtedly contribute to the differences in the activity of invertase in various parts of the stalk, it is suggested that the differences in percentage of potassium are important. Further studies along these lines are now under way.

Certain data in table VIII require discussion at this point. There it will be seen that the invertase of the stems of series 2, April material, is less active than that of series 1 or 3, whether buffered or not. This puzzled the writer until the distinction between green and dry leaf cane became apparent. The plants of series 1 and 2 were very much larger than the others and consequently had considerably more dry leaf cane. Inasmuch as the entire sticks were compounded, the weak invertase of the dry leaf cane would naturally decrease the figures for the entire stalk. This may also explain why the activity of the stems of the controls is so much less in the April than in the November harvest, there being considerably more dry leaf cane in April than in November. The distribution of invertase throughout the cane plant is now being studied and will be reported in a later contribution.

The sensitivity of the invertase of yeast to hydrogen ion concentration is well known and has been discussed by FALK (**15**). The data presented in table VII show that the invertase of sugar cane is similarly affected, having in these plants an optimum reaction very close to that of the invertase of yeast. The effect of hydrogen ion concentration upon the activity of invertase differs from its direct effect upon inversion: in the former case there is a definite optimum around pH 4.4, the activity falling off at other reactions; in the latter case inversion increases with increasing acidity as far as has been tested. The significance of this situation has been examined by FALK, who suggests that the hydrogen ion and the enzyme may attack sucrose differently.

The hydrogen ion concentration of the expressed sap of the blades and stems of the plants harvested December 4 was pH 5.2 to 5.3 (table I), while

the optimum reaction for the invertase of the plants harvested November 20 was pH 4 to 4.4 (table VII). The question arises as to the effect of the hydrogen ion concentration of the expressed sap of cane upon the quality of the juice. In Trinidad, FOLLETT-SMITH (**18**) found the sap of cane leaves to be pH 5.1–5.3. So far as the writer is aware, these are the first determinations to be made in Hawaii of the hydrogen ion concentration of the expressed sap of sugar cane. It is therefore impossible at present to compare these results with others in Hawaii. Some data are available for the reaction of crusher juices at the Honolulu Plantation Company, where the average of 50 determinations is pH 5.17 for crusher juice and pH 5.29 for mixed juice. Although these figures are close to those obtained in the present study, probably little value should be assigned to them in this connection for two reasons: (1) the crusher juice is contaminated with soil and fertilizers; and (2) the crusher juice is composed largely of the juice from dry leaf cane, which may or may not have the same reaction as green leaf cane. There is need, therefore, for further studies concerning the hydrogen ion concentration of the expressed sap, invertase activity, and juice quality. Several questions arise. Is the optimum reaction for the formation of sucrose the same as the optimum for the analytic activity of invertase? If the synthesis of sucrose is as sensitive to reaction as the activity of invertase is shown to be in this paper, which seems likely from the work of OPARIN and KURSSANOW (**39**), then studies should be made of the possibility of controlling the hydrogen ion concentration of the cell sap of sugar cane.

The invertase studies reported here show that the following factors affect its activity: potassium, hydrogen ion concentration, and location in the plant. Inasmuch as enzymes are very sensitive to conditions, it seems likely that many other climatic and edaphic factors may affect its activity. The evidence presented in this paper would seem to indicate that invertase in the sugar cane plant synthesizes sucrose, and that where invertase is most active the greater synthesis of sucrose occurs. For the past 20 years the increase in sugar percentage within the cane plant has not been commensurate with the increase in the applications of fertilizers, although the increase in the total sugar produced per acre has been large because of the greater size of the plants, a point which has been discussed by MARTIN and MCCLEERY (**35**). An important problem in the sugar industry today, therefore, is the improvement of the quality of the juices. Juice quality is known to be affected by weather, fertilizers, etc., but the mechanism is not fully understood. It would seem that both external and internal factors may affect the activity of invertase and that a study of these may aid in controlling the production of sugar. Such studies are now under way.

Another important effect of potassium deficiency in sugar cane is the increase in the activity of amylase. A relationship between potassium content and amylase activity has been suggested by several investigators. ROBBINS (**42**) studied the secretion of diastase by *Penicillium camembertii* and concluded that there was no relationship between potassium and diastase formation. ENGLIS and LUNT (**13**) reported on the diastatic activity of the nasturtium; they found that the activity of diastase decreased with increased potassium in sand, but in peat the medium application of potassium gave the highest activity. DOBY and HIBBARD (**10, 11**) found increased diastase activity in sugar beet plants deprived of potash. JAMES (**28**), from his studies of the effect of potassium upon photosynthetic efficiency in the potato, postulates that potassium increases the activity of diastase. Although for other plants the results are not always in agreement, a more active amylase in potassium-deficient plants seems now to be well established for sugar cane, since the present results are in accord with the former (**24**).

For the sake of clarity, most of the discussion of the amylase determinations was incorporated with the presentation of results and the reader is referred to that section at this point. The following possibilities arose as explanations of the increased amylase activity in the potassium-deficient plants: the quantitative regulation of amylase production by sugars; the stimulating effect of phosphorus; a direct regulatory or protective effect exerted by potassium. Certain theories almost certainly disproved were mentioned in the results and will not be repeated here.

Although some evidence was obtained in favor of the theory of the quantitative regulation of enzyme production by the substrate, that theory is not of general application in the present study because of the negative relationship between the amylase activity and percentage of reducing sugars in the stems of the plants harvested in April, whereas the correlation should be positive to uphold the theory.

The greater amounts of phosphorus in the potassium-deficient plants may play a rôle in their amylase activity, since in both the November and April material the largest amounts of phosphorus occurred in the plants deficient in potash, as shown in tables XV and XVI of the first report in this series (**26**). The correlation between phosphorus content and amylase activity is not perfect, however, and the equalization of the phosphorus in the blades of the November and April material did not result in equal activity of amylase. It is interesting, on the other hand, that the amylase of the potassium-deficient plants was activated by the addition of more phosphorus, while that of the control was not. The plants deficient in potassium contained more phosphorus and their amylase was more readily activated by phosphorus. This is indeed an important factor.

On the whole the evidence at hand seems to favor most strongly the idea that potassium acts as a regulator or protector of the enzyme amylase. It must be considered that the activity in the control plants is "normal" while that in the plants deficient in potassium is "abnormal." The suggestion of GOODWIN and HANGER (**20**) is pertinent, that amylase is a negatively charged ion. If so, it might readily unite with the positively charged potassium ion and this might explain the dialysis results. Dialyzing one day might remove the inorganic activators and thus decrease activity, but further dialysis (3 days) might cause or allow the amylase-potassium compound to dissociate, the potassium going through the membrane, leaving the amylase unprotected and thus more active. Possibly the negatively charged amylase is more readily hydrated than the amylase-potassium compound, since the charging of proteins is known to run parallel with hydration. This might aid in the digestion of starch, the first step of which is hydration. Further evidence of the protective effect of potassium is found in the fact that the amylase activity of the potassium-deficient plants was more readily activated by phosphorus than was that of the controls. DOBY and HIBBARD (**10, 11**) also found that the diastase of sugar beets deficient in potassium was activated by salts more readily than that of beets supplied with an adequate amount of potash. There is a negative relationship between potassium and amylase in both the blades and the stems of the material harvested in November as well as in April (table XV). Changing representative plants from solutions lacking potassium to the control solution in January resulted in a decrease in their amylase activity (table XIV). In short, the evidence points to the view that one function of potassium in the sugar cane plant is the regulation or "protection" of the enzyme amylase. Leaf diastase is supposed to consist of an inactive pro-enzyme and an activating co-enzyme, according to WAKSMAN and DAVISON (**53**). Possibly potassium exerts a protective action on the pro-enzyme, thus preventing its activation by the co-enzyme or other accelerators.

The condensations of dextrose in the formation of dextrins and starch tie up the sugar in undesirable forms which may result in decreasing the yield of sucrose. Starch occurs in cane as a temporary storage product in the starch sheaths of the leaves. This starch is probably for the most part digested to sugar and translocated to the stems and stored as sucrose, in the ordinary course of events. In certain varieties, however, a deposition of starch occurs in the stems, particularly in the nodes. This is especially true of Uba canes, a condition which has been studied by FEUILHERADE (**16, 17**), HADDON (**23**), VON STIEGLITZ (**44**), and WELLER (**54**). One is led to wonder whether the amylase activity of Uba is different from that of other varieties.

The production of too much starch and the like in cane is undesirable not only because of the utilization of dextrose which should go to the formation of sucrose, but also because the presence of certain gums, dextrins, and other higher carbohydrates in the juices leads to difficulties in the process of clarification, as brought out by FEUILHERADE and STIEGLITZ.

Amylase and invertase differ in several respects and a consideration of these differences is interesting. Amylase was more active in the potassium-deficient plants whereas invertase was less active. Amylase was found to be much less sensitive to hydrogen ion concentration than was invertase. Invertase was activated by potassium but not by sodium, whereas amylase was activated by everything tested. The two enzymes behaved similarly in that both were more readily activated in the potassium-deficient plants than in the controls.

RÔLE OF POTASSIUM

The question of the rôle of potassium in the nutrition of plants has interested plant physiologists for years, and men have sought to assign one particular function to the element, *e.g.*, photosynthesis, protein synthesis, translocation, or others. The research of recent years, (**3, 21, 24** and **26, 28** and **29, 37,** and others) has shown that the problem is not so simple, but that potassium probably affects directly or indirectly many if not all of the cellular activities of plants. Thus the present studies show that sugar cane plants deficient in potassium may develop the following characteristics: low percentage of moisture; high total ash content when young, followed later by low ash percentage; high percentages of calcium, magnesium, phosphorus, and iron at certain ages; derangements in the synthesis and translocation of proteins and sugars; phloem necrosis; accumulations of iron at the nodes; weak activity of invertase; and strong amylase activity. Undoubtedly other derangements occur. The total result of these conditions is the cessation of growth of the entire plant, discoloration of the leaves, and dieback of the leaf tips, usually mentioned as the symptoms of potash starvation. The writer is still of the opinion that these derangements are concatenated, as proposed previously (**24**). One of the earliest occurrences in potassium starvation is the increased absorption of other ash constituents, notably phosphorus, calcium, and magnesium, which may enter the plant more readily in the absence of the more mobile potassium. Because enzymes are sensitive to conditions, being readily activated and inactivated, they are soon affected by the lack of potassium. Amylase, not being protected by potassium, is more readily activated by the greater amount of phosphorus, which may cause the accumulation of starch which has been found in some plants. Invertase, peptase, reducase, and probably other enzymes either fail to develop or lose their activity. In the present study

invertase activity was weak, possibly due to the lack of activation by potassium. The weak activity of invertase led to derangements in the transformations between the reducing sugars and sucrose. Derangements in the formation of proteins were also found. The greater amount of iron which entered the potassium-deficient plants accumulated at the nodes, possibly because of a more sluggish transpiration stream. Necrosis of the phloem, which occurred probably both because of the accumulations of iron and because of the derangements in the synthesis of proteins, led to a decrease in the translocation of both sugars and nitrogenous compounds. This eventually caused the stems to be low in reducing sugars, sucrose, total and amino nitrogen. The decrease in the absorption of ash constituents found at the end of the experiment was only to be expected, the poor absorbing capacity of the roots being shown by their weak and discolored condition. Probably all of these factors contributed to the development of the external symptoms.

The properties of potassium by which it functions are probably not one but several. ANDRÉ and DEMOUSSY (**1, 2**) have stressed its mobility which, as was shown in the first paper of this series (**26**), may help explain its migration out of the dying leaves. Also the decrease of the mobile potassium may cause the increase in the absorption of other ash constituents. The suggestion of LOEB (**33**) that the selective absorption of potassium by plants is due to its being built up into complex compounds has been disproved by KOSTYTSCHEW and ELIASBERG (**32**), who found that potassium could be extracted *in toto* by water.

LOEW (**34**) found that potassium has special condensing abilities which sodium lacks. Considerable work has been done by STOKLASA and coworkers (**45–49**) as well as by ZWAARDEMAKER (**56**) to show that the radioactive properties of potassium are important physiologically, whereas BURKSER, BRUN, and BRONSTEIN (**7**) found no evidence of bioradio-activity in the plants they studied.

The writer cannot subscribe to the suggestion that the photoelectric effect of potassium is of physiological significance, as recently proposed by BRUNO (**6**) and JACOB (**27**). Although it is true that potassium is one of the few elements which exhibits the photoelectric effect in ordinary daylight and that it is slightly photoelectric even at 700 μμ (**41**) (thus throughout the region of the spectrum which is chiefly absorbed by plants and where photosynthesis occurs), yet a serious difficulty prevents this from being of significance in the physiology of plants. In order for potassium to exhibit the photoelectric effect in plants it is essential that some of the element be present in colloidal metallic form, because it is the valence electrons which are emitted in the photoelectric effect. If these are already oxidized in a bond with an acid radical none will be free to be emitted photoelectrically. Be-

cause all parts of plants contain water in which potassium readily oxidizes, the presence of colloidal metallic potassium in plants seems impossible.

Unless some way is found of surmounting this difficulty, it appears that the photoelectric properties of potassium cannot be used in explaining its physiological activities in plants. The fact that symptoms of potash deficiency develop in bright light more quickly than in dim light (**26**) may be just a question of limiting factors.

Because of its mobility, its special condensing properties, its radioactivity, and probably also other properties, it seems likely that potassium affects either directly or indirectly most of the activities of plants, and it is at present impossible to assign one particular process as the special rôle of potassium in the physiology of plants.

Summary

1. This paper reports the results of determinations of the enzymes invertase, amylase, and ereptase; analyses of total and amino nitrogen, reducing sugars and sucrose; and certain physico-chemical determinations of the juices expressed from the leaves, stems, and roots of sugar cane plants supplied with varying amounts of potassium.

2. Very little difference was found between the hydrogen ion concentration, titratable acidity, and buffer systems of the expressed juices of the controls and the plants deficient in potassium.

3. The effect of potassium deficiency upon the relative amounts of protein and amino nitrogen in the plants varied with their age.

4. Two months after starting the plants in the nutrient solutions there was a higher percentage of amino nitrogen and a lower percentage of protein nitrogen in the blades and the stems of the plants starved for potassium than in the controls.

5. Seven months after starting the plants in the nutrient solutions higher percentages of amino, protein, and total nitrogen were found in the blades of the potassium-deficient plants, while in the stems the opposite relationship was found to occur.

6. The conclusion is drawn that both the synthesis and the translocation of proteins are diminished by potash starvation.

7. The curtailment in the synthesis of proteins was found to occur after the formation of amino acids rather than before, indicating that in these plants the reduction of nitrates proceeded as usual.

8. The suggestion is made that the interference with the translocation of proteins and carbohydrates is caused by a necrosis of the phloem.

9. The blades of the plants deficient in potassium contained higher percentages of reducing sugars and lower percentages of sucrose than the controls. The percentages of total sugars in the blades remained about the same.

10. The relationships between the sugars in the stems depended upon the degree of potassium deficiency. The stems of the plants partially starved for potassium (series 2) contained higher percentages of reducing sugars and lower percentages of sucrose than the controls; while the stems of the plants more completely deprived of potassium (series 4 and 5) were very low in reducing sugars as well as sucrose. There was a positive correlation between the amount of potassium supplied and the total sugar stored.

11. The conclusion is drawn that potassium deficiency results in derangements in the transformations between the hexoses and sucrose, as well as in curtailment of their translocation.

12. No evidence of the accumulation of carbohydrates in the plants deficient in potassium was obtained in this study.

13. The optimum reaction for the activity of invertase in the blades and the stems was found to be around pH 4.4.

14. The invertase activity of the plants deficient in potassium was weaker than that of the controls, in both blades and stems, when tested unbuffered. The invertase activity in stems was affected more severely by potassium deficiency than that in blades.

15. When tested at the optimum reaction, the invertase activity was equal in all the blades. The activity in the stems was not equal at the optimum reaction, although the difference between the extremes was less than when tested unbuffered.

16. Because the invertase activity in all the blades was equalized at the optimum reaction, it seemed that potassium is not essential for the formation of invertase.

17. Because the addition of potassium phosphate to the stem material of series 5 increased the activity of invertase while the addition of sodium phosphate to series 1 had no effect, it is concluded that potassium is a specific activator for invertase in sugar cane.

18. In a supplementary experiment, in which variety P.O.J. 2878 was used, the invertase activity of the green leaf portion of the stalk was double that of the dry leaf cane.

19. These studies offer indirect evidence that sucrose in the sugar cane plant is synthesized by invertase.

20. The suggestion is made that determinations of the activity of invertase might aid in distinguishing between plants in the field which, like the plants of series 1 and 2, are very much the same in color, size, and general condition but which show a difference in the amount of sucrose in the stems.

21. The optimum reaction for the amylase of blades was found to be pH 5.9. The amylase of stems seemed not to be much affected by the hydrogen ion concentration.

22. Amylase activity was greater in the plants deficient in potassium than in the controls, in both blades and stems.

23. Studies were made of the effects of potassium, phosphorus, calcium, magnesium, dialysis, and sugars upon the activity of amylase.

24. Several possible causes of the increased activity of amylase in the plants deficient in potassium are examined and the conclusion is suggested that potassium deficiency removes the protective action of potassium from amylase, thus allowing the enzyme to be activated more readily by phosphorus, which is present in potassium-deficient plants in greater amounts than in the plants supplied with an adequate amount of potash.

25. The increased activity of amylase in the plants starved for potash is an undesirable characteristic because under the conditions of condensation in the stems it might lead to the formation of starch. This is undesirable both because of the utilization of dextrose which should go to the formation of sucrose and because the presence of dextrins and certain other higher carbohydrates in the juices leads to difficulties in the process of clarification.

26. The optimum reaction for the activity of ereptase in the blades and roots was pH 4.9, and in stems, pH 5.9.

27. Potassium seemed to have no effect upon the activity of ereptase, with the exception of the roots, in which the greater activity occurred in the plants deficient in potassium.

28. Attempts were made at several reactions to determine the activity of peptase, using the carmine fibrin method of GRÜTZNER and the titration method of WILLSTÄTTER and WALDSCHMIDT-LEITZ. No peptase activity was detected.

29. The writer is still of the opinion that the derangements in the physiology of sugar cane deficient in potassium are concatenated, as proposed previously. An outline of the concatenation of these derangements is suggested.

30. The properties of potassium by which it functions are considered. Regarding the suggestions recently appearing in the literature that the photoelectric properties of potassium may be of physiological importance in plants, the difficulty is pointed out that this would require the presence of colloidal metallic potassium in plants, whereas the universal distribution of water in plant tissues would render this impossible owing to the ready oxidation of potassium.

31. It seems likely that potassium affects either directly or indirectly most of the activities of plants, and it is at present impossible to assign one particular process as the special rôle of potassium in the physiology of plants.

EXPERIMENT STATION, H. S. P. A.
HONOLULU, T. H.

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GERMINATION OF THE RED SPRUCE

HENRY IVES BALDWIN

(WITH FOURTEEN FIGURES)

Introduction

Since forestry first became a subject for discussion in northeastern America, no problem has received attention longer, nor so frequently, as the question of the natural reproduction of the spruce and fir forests of the northeastern United States and southeastern Canada. Interest was early drawn to it by the pioneer forestry work of GIFFORD PINCHOT and HENRY S. GRAVES in the Adirondacks in 1895–1896, and by the extraordinary growth of the paper and pulp industry based on the utilization of these forests. Until recently this utilization has continued at an ever-increasing rate, faster indeed than progress toward solving the problems of forest replacement.

A great industry and the prosperity of a large section of northern New England and New York are dependent on the perpetuation of spruce and fir in the forest. While hardwoods are being pulped to some extent, and economic surveys point to a shift of some branches of the paper industry, like the lumber industry before it, to the south and Pacific northwest, yet for certain qualities of pulp New England spruce will doubtless always be preferable (**8**). Without the admixture of spruce and fir, the forests of northern hardwoods would have far less economic value and would lose much of their charm for the tourist, and the wooded mountain sides would be less effective regulators of stream flow.

In the 35 years since spruce reproduction was first studied systematically in this country, knowledge of the factors affecting it has increased slowly. Our understanding of the spruce-fir forest environment is vastly greater, but little has been published on the germination of red spruce (*Picea rubra* Link.). A study of natural reproduction by seed should start with consideration of the seed. With few exceptions this has not been done. Without a thorough knowledge of the seeding habits of the species and of the behavior of the seed, as influenced by various environmental factors, however, a true understanding of the phenomena of establishment cannot be acquired. Only when the most favorable conditions for germination are known can the silviculturist plan cutting methods best calculated to secure these conditions.

The investigations reported here are studies of seed behavior. They do not attempt to go beyond the germination process itself. It must be conceded, however, that the success of spruce reproduction is often as much or more dependent on factors affecting survival during the critical first months

following germination, or even during the first few years, than upon germination itself. It is planned to deal with the survival and establishment of seedlings more fully in a later publication.

Historical survey

The literature on seed germination is so extensive that the present review is restricted chiefly to that concerning red spruce. KORSTIAN (**30**) has recently reviewed the literature on seeds of broad-leaved trees; HAASIS (**19**) has dealt with the special subject of temperature coefficients of the germination of coniferous seeds; and JOSEPH (**24**) and BARTON (**5, 6**) have pointed out the importance of after-ripening in many arborescent species. Finally, TOUMEY and STEVENS (**58**), SCHMIDT (**49**), and TOUMEY and KORSTIAN (**56**) have brought together much of the existent information on forest tree seed.

Careful accounts of the germination of red spruce seeds under natural or artificial conditions are comparatively scarce, and for the most part, recent. The first observations appear to have been made in America. Many papers might be cited, few of which describe germination itself. PINCHOT (**41**) published the first silvicultural study of red spruce. He states that the seeds, "where there is light enough, germinate and grow easily on deep spruce or pine duff, and on heavy beds of moss, . . . a heavy matting of leaf litter . . . is less thoroughly adapted to their requirements." GRAVES (**18**) makes a similar statement regarding germination. The ideas about the importance of light which prevailed at the beginning of the century have gradually changed as the decisive rôle of soil moisture has become better appreciated. CHITTENDEN (**13**) believed red spruce seeds do not germinate as readily as those of balsam fir (*Abies balsamea* (L.) Mill.) and are more exacting in regard to seed bed. He recognized, however, that no special light intensity is required for germination. MURPHY (**35**) compared the establishment of the two species in detail. He stressed the fall germination of spruce as a factor placing it at a disadvantage with fir. During the autumn he believed the soil moisture and other factors to be less favorable for the germination of spruce in the open than in the forest. Assuming that the chief seed distribution in the open occurred later in the winter, he concluded that the percentage of spruce seed which would lie over for spring germination would be greater in the open than in the forest. In a later publication (**36**) he emphasized the importance of soil moisture in controlling germination. The heavy mat of hardwood leaves under hardwood stands often prevents the seed from reaching moist soil, or, germination having taken place, the seedlings are unable to emerge, and succumb to mold under the poorly aerated mulch. A similar explanation was given by MURPHY and ZON (**37**).

MOORE (**32**) experimented to determine whether red spruce seeds remained alive when stored in the duff. He transferred flats of undisturbed humus, dug up in the dense forest, to an opening and watered them. Some seeds were sown in the corners of the flats. The results were inconclusive, since it was uncertain whether the seeds which germinated were ones stored naturally in the duff, or artificially sown. In another case (**33**) he grew seedlings of spruce and fir in flats filled with various soils. The spruce grew best in mild humus, but withstood high acidity better than fir. In a further study he states: "Germination occurs directly in the humus, contact with the mineral soil seeming unnecessary." He considered hardwood leaves unfavorable, not only hindering penetration of the spruce roots, but also preventing the shoots from coming to the surface. He thought it probable that spruce seedlings germinated and grew between the leaves a certain distance, but were unable to push through them. WESTVELD (**61, 62**) has recently carried out experiments on seed-bed conditions for red spruce. Seed was sown in the fall on the undisturbed litter and on plots cleared of litter. On June 11 the following spring there was an abundance of spruce seedlings on the cleared plots, but none on the undisturbed litter. By fall, however, a number of spruce seedlings had appeared on the undisturbed plots as well as an increase of 75 per cent. on the cleared plots. This would seem to indicate that even after favorable conditions for after-ripening during the winter, there is considerable delay in the spring germination on litter. Judging from the promptness with which germination occurs under optimum conditions with dry or after-ripened seed, it seems likely that these results were due to fluctuations in soil moisture, or that additional seed was shed during the summer.

There is considerable scattered information on the size, quality, and germination of red spruce seed as the result of laboratory tests. Red spruce cones give about the same yield of seed as many other conifers. TOUMEY and KORSTIAN (**56**) give the average as 1.03–1.04 lb. clean seed per bushel of cones. STETSON (**50**) found an average of 8843 seeds per oz., or 3.06 gm. per 1000 seeds. RAFN (**42**) gives 3.6 gm. per 1000 (only one sample tested). TOUMEY (**53**) gives the average number of clean seeds per pound as 131,400, or 3.16 gm. per 1000. JACOBSEN (**23**) gives the following weights per 1000 (in gm.):

Highest 3.4	Lowest 2.8	Average 3.1

TOUMEY and KORSTIAN (**56**) quote TILLOTSON (**52**) as finding 130,000 seeds per pound, and BUELL (**11**) 139,200 for North Carolina seed. There would thus appear to be a possibility of some difference in weight of seed in different parts of the tree's range. Ordinarily the heaviest seeds are borne in the more southerly parts of the range.

The same investigators give some scanty information on germination. SUDWORTH (**51**) gives the germination of fresh seed as 60–70 per cent.; STETSON (**50**) obtained 25 per cent. with 1909 seed and FAHRENBACH (**17**) but 6 per cent with 1910 seed. RAFN (**42**) reported 31.7 per cent. in 30 days from one lot, and JACOBSEN (**23**) lists the average of three lots as:

High 76 per cent. Low 68 per cent. Average 71 per cent.

Her tests were continued 28 days on the JACOBSEN apparatus at 16–28° C. alternating temperature. TOUMEY (**53**) and TOUMEY and KORSTIAN (**56**) give 54 per cent. as the average in soil tests. MURPHY (**36**) states that "50–90 per cent. of the seed are perfect, and of these 60–80 per cent germinate."

Few exact studies of the germination requirements for red spruce appear to have been made. Forestry and ecological literature abounds with general statements about the most favorable seed-bed, and with theories of the requirements for acidity or light. Observations of fall germination suggest that prolonged after-ripening is not required. Moisture seems to have been found the outstanding factor influencing germination.

Investigation

Description of red spruce seed

Mature red spruce seeds are very dark brown, about 3 mm. long, with short broad wings rounded above the middle. The testa, or coat, consists of an outer crustaceous layer, dark brown and occasionally mottled or streaked, and an inner membraceous layer, pale chestnut brown and lustrous. The pale yellow embryo is axile in a large fleshy endosperm. The number of cotyledons varies from four to seven. While slightly larger, the seeds of red spruce are often difficult to distinguish from those of black spruce (*Picea mariana* (Mill.) B.S.P.) and from white spruce (*Picea glauca* (Moench.) Voss) when the wings are lacking. Illustrations of the seeds are given by SARGENT (**47**).

Factors affecting germination

The phenomenon known as germination consists of a renewal of activity of the embryo, much as the vegetative parts of a tree begin to grow again after a resting period. The criteria by which one judges whether germination has taken place are largely arbitrary. BRANDTS (**9**) has suggested that germination be considered to take place when the first new cell divisions occur in the formative region of the root. Generally, however, emergence of the radicle or hypocotyl, so that it projects beyond the edge of the testa, is accepted as a criterion of germination. In soil tests emergence of the seedling above ground is so accepted.

In the developing embryo, as in the branch bud, both external and internal factors are operative in causing renewed activity. The internal fac-

tors are those inherent in the seed, or such causes as dormancy, size and weight of seed, age, degree of maturity, and hereditary characteristics. CROCKER (**14, 15**) has classified the causes of dormancy, and shown that in some species delayed germination is occasioned by a dormant embryo; in others, by inhibitory seed coats or combinations of these factors. In seeds capable of immediate germination, external factors such as temperature, moisture supply, and oxygen supply are the chief controls of germination. Light is rarely of importance in the germination of tree seeds, and the greatest influence of solar radiation is in its effect on temperature and soil moisture, as shown by BOERKER (**7**). Soil acidity and carbon dioxide concentration no doubt affect germination in nature, either directly or indirectly. Of the possible rôle of microorganisms little is known.

CHARACTER AND SCOPE OF INVESTIGATIONS

The investigations undertaken consisted partly of field and partly of laboratory experiments.

I. FIELD EXPERIMENTS.—The field work was begun as part of an investigative project for the Brown Company on the Cupsuptic Experimental Area in Adamstown, Maine, in the fall of 1924. Experiments were later continued and augmented by plots established on the Dummer Experimental Area, Dummer, New Hampshire, in 1925, and on the Jerico Forest, near Berlin, New Hampshire, in 1926. These experiments were planned and executed with the following objectives:

1. To determine the rate of germination and survival of spruce under different soil conditions. A large number of small seed plots were prepared and sown with spruce seed. Some were covered with spruce branches, some with wire cages to exclude birds and animals, and some were left open and unprotected. Germination and survival data were obtained by counts at intervals. A brief summary of these results is given only in so far as they touch upon the present problem.

2. To determine the rate of establishment of natural spruce reproduction on quadrats subjected to special treatment:

   (a) Exposure of quadrats denuded of humus, herbaceous and woody shrubs, or by combinations of all these treatments.

   (b) Comparison of trenched and untrenched quadrats to demonstrate the influence of competition for soil moisture and nutrients.

3. To determine the length of retention of vitality of seed stored in the duff by:

   (a) Testing seeds taken from duff samples under various conditions of site and forest type.

   (b) Testing seeds stored under cages in insect-proof copper wire boxes buried in the duff on various sites.

II. Laboratory experiments.—The laboratory work comprised the following experiments on red spruce germination under controlled conditions, so far as practical to attain, in order to determine the effect of the various factors on germination. Experiments were conducted on:

1. The moisture and ash content of the seeds used in the experiments.
2. The average germinative energy of seeds stored for different periods.
3. Germination at different constant temperatures.
4. Moisture supply: relation of water absorption to germination.
5. Germination in light and darkness.
6. After-ripening treatment.
7. Oxygen supply: germination under water with and without aeration.
8. Germination in different hydrogen-ion concentrations.

Material

Red spruce seed collected in northern New Hampshire was used in these experiments. It was extracted at approximately 50° C., winged and cleaned dry at the Brown Co. Seed Extracting Plant at Berlin, New Hampshire. In the field work seed collected in 1924 and 1925 was used; for the laboratory experiments seed of the 1927 and 1928 commerical collections was used. It was stored air-dry in sealed tins at approximately 15° C. until June, 1929, in the case of the 1927 seed, and October, 1929, in the case of the 1928 seed. From then until placed to germinate it was kept in tightly corked glass bottles at room temperature, or in a cellar at approximately 15° C. In some of the last tests similar seed from the 1929 crop was used.

The 1927 lot was found to weigh 3.222 gm. per 1000 filled seeds. Empty seed weighed 37 per cent. as much as filled seed. The 1928 lot contained 7.8 per cent. moisture, and the ash was 4.7 per cent. of the dry weight. In the laboratory work 70,000 counted seeds were used. Most experiments were run with eight duplicate samples of 100 seeds each, for each variable condition.

Experimental methods and results[1]

I. Field experiments

1. Germination and survival on seed plots.—In connection with direct seeding experiments, observations were made on germination and survival under various conditions of soil and cover. The experiments were made in 1925 at Cupsuptic Lake, Maine, and in 1926, 1928, and 1929 at Berlin, New Hampshire.

Plots about 30 cm. square were prepared with a grub hoe by stripping off the sod or upper layers of humus. Seeds were sown and gently pressed

[1] Source tables are omitted on account of lack of space. They may be consulted in bound copies of the original manuscript on file in the Yale University Library.

into the soil, or at times lightly covered. In many cases 100 seeds were sown; in others, less. At the time of sowing, notes were taken on the character of the soil in each plot. Some plots were protected with spruce and fir branches, and about 60 with small wire cages to prevent molestation by birds and animals. Alternate plots were left unprotected. Each plot was designated by a small numbered stake to make relocation certain. Plots were examined and seedlings counted once or twice a year. In the different experiments about 1000 plots were studied.

In all cases more seedlings were obtained under the wire cages, owing possibly to conservation of soil moisture by the sides of the cage as much as to protection from birds. In the later experiments about 100 per cent. more seedlings appeared in the protected plots than in the unprotected ones. Sand or mineral soil proved better than humus or rotted wood as a seed-bed, confirming previous conclusions by many observers of red spruce as well as those of BARR (**4**). Germination and survival were higher when the seed spots were protected by spruce boughs than when uncovered and exposed. These shaded the surface soil from direct sunlight and prevented loss of moisture, especially after the needles dropped off and formed a mat on the soil. It has been repeatedly shown that freshly germinated tree seedlings can be killed by high surface soil temperature. The best seed-bed as indicated by these experiments would be found where moisture is available at all times, and where there is adequate protection against surface temperatures above 50° C. (TOUMEY and NEETHLING, **57**.)

2. NATURAL SEEDING AND ESTABLISHMENT AS RELATED TO SOIL CONDITIONS. —The results of natural seeding on denuded quadrats were rather disappointing, since very few seedlings appeared. This was attributed partly to the poor seed supply where the plots were located, and partly to the destruction of seed by various agencies.

In the fall of 1925 a plot two milacres in area under a fully stocked stand of mature red spruce in Dummer, New Hampshire, was trenched, severing all roots entering the plot to a depth of 0.5 m. Trenching was repeated in 1926 and 1928. An adjacent untrenched plot was established as a control. Spruce and fir seedlings and other vegetation on the plots were mapped and photographed at intervals. New seedlings which germinated were designated by colored toothpicks. At the start the vegetation on the two plots was practically identical, but after two years the tree seedlings, herbs, and mosses were noticeably larger and more abundant on the trenched plot. It is not known whether each plot received the same quantity of seed, but germination and survival were much greater on the trenched plot, owing chiefly to the more abundant soil moisture. Mention is made of these plots by TOUMEY and KIENHOLZ (**55**).

3. RETENTION OF VIABILITY BY SEED STORED IN DUFF OF FOREST FLOOR.—The rapid establishment of seedlings of some trees on recent cuttings or burns where there are no seed trees in the vicinity or when there has been no seed crop recently has led foresters to conclude that such reproduction arose from seed stored in the duff. HOFMANN reached this conclusion, since largely disproved in the case of Douglas fir by the investigations of ISAAC (**63**) and HAIG (**20**). CARY (**12**) states that seed stored in the duff must be relied upon for reproduction of Sitka spruce (*Picea sitchensis* (Bongard) Carrière) in Alaska. The possibility of spruce seed being stored in the duff in eastern forests has been suggested by MULLOY (**34**) and others, but few definite experiments have been made.

Two methods were used for investigating viability of seed stored in the duff. (a) Samples of duff were analyzed and germination tests applied to seed recovered. (b) Seeds of known germinative capacity were sown under the litter, or inclosed (thoroughly mixed with duff) in fine-mesh wire cages and buried in the duff. After remaining buried a known length of time, the cages were dug up and the seeds recovered and tested for germination, in comparison with seeds of the same lot stored in the laboratory. The first method suffers from several objections: it is impossible to determine the age of the seed recovered; it may be freshly fallen; its original germinative capacity and hence its deterioration in storage are unknown. Results of duff sampling depend very largely upon the location, the proximity of seed trees, and the density of the local rodent and bird population, which might consume a considerable amount of the best seed. The second method overcomes some of the disadvantages of the first, while open to the objection that the method of storage is slightly less natural.

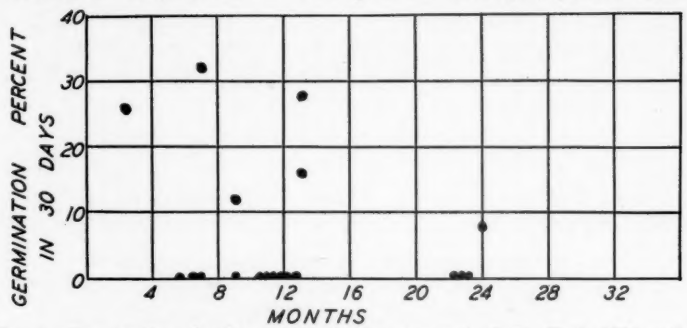

FIG. 1. Germination of red spruce seed after storage in duff. Basis 3200 seeds.

(a) *Analysis of duff samples.*—The first samples were taken at Cupsuptic Lake in the summer of 1925 before the seed of the year was ripe. Later, samples were taken in the autumns of 1925 and 1926 in Dummer,

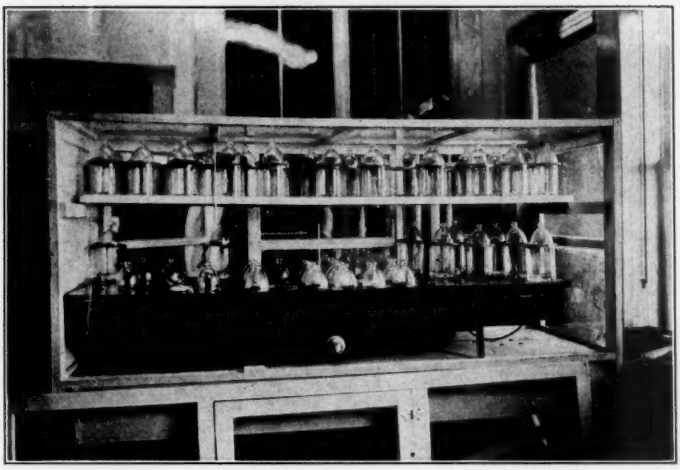

FIG. 2. JACOBSEN type germinator with front of case removed, showing also individual JACOBSEN germinators.

New Hampshire. An area 30 cm. square was laid out and a section 10 cm. deep was cut and lifted out with a large machete. It was brought into the laboratory and dried for several weeks at room temperature in a tray

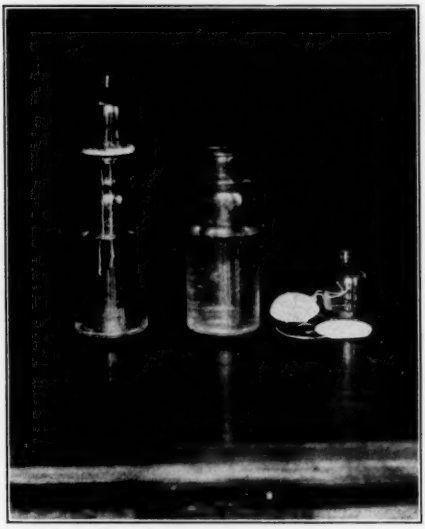

FIG. 3. Individual JACOBSEN germinators.

TABLE I

ANALYSIS OF DUFF SAMPLES, SIZE 30 × 30 × 10 CM.

SAMPLE NO.	DATE LIFTED	DESCRIPTION OF DUFF	LOCATION	FOREST TYPE	CROWN DENSITY	EXPOSURE	NO. OF SOUND SPRUCE AND BALSAM SEEDS RECOVERED	GERMINATION
								%
1	Sept. 8 1925	*Hylocomium* moss, bottom dead moss, and rotted wood	Plot S-3 Cupsuptic Lake, Me.	Pure spruce knoll, cut clean 1924–5	0.0	W	174	0
2	Oct. 16 1925	Spruce needles in all stages of decay	Plot B-25 Dummer, N. H.	75-yr. pure spruce	1.0	S	460	0
3	Nov. 17 1925	Beech and sugar maple leaves	Near R-35 Dummer, N. H.	Old growth, hardwood, 100 m. east of spruce	0.8	E	17	0
4	Oct. 14 1926	Spruce needles	Plot B-25 Dummer, N. H.	75-yr. pure spruce, edge of stand	0.5	W	300 +	0
5	"	Spruce needles	Plot B-25 Dummer, N. H.	75-yr. pure spruce, center of stand	1.0	S	38	0
6	"	Beech and maple leaves	Near R-35 Dummer, N. H.	Culled virgin hardwood	0.7	E	4	0

screened against rodents and insects. When thoroughly dry, the sample was rubbed through wire screens of progressively finer mesh until the seeds were caught. The seeds thus obtained were counted and tested for germination. Duff samples from locations where seeds had been shed recently, as the cut-over sites at Cupsuptic Lake, contained abundant seeds, as shown in table I. All attempts to cause these seeds to germinate failed, however. Six samples of these seeds were submitted to the Boyce Thompson Institute for Plant Research for testing. From these only one seed germinated. Most of the others seemed to have been attacked by fungi (table II).

TABLE II

TESTS OF SPRUCE SEED RECOVERED FROM DUFF SAMPLES*

No. of sample	No. of seeds	No. without embryo	No. with embryo	No. lighter than water (of seeds with embryo)	No. heavier than water	No. germinated
1	76	54	22	22	—	—
2	3	1	2	2	—	1
3	14	9	5	5	—	—
4	195	140	55	53	2	—
5	36	31	5	5	—	—
6	74	73	1	1	—	—

* Tests made by Boyce Thompson Institute, January, 1926. All seeds except no. 2 were heavily attacked with mold from the start. All seeds had the seed coat cut open in one place to examine for presence or absence of embryo. They were sterilized with 0.25 per cent. uspulun for 5 minutes before being placed to germinate.

(b) *Burying seed samples.*—In burying seed for subsequent digging up and examination, use was first made of frames 1×1.5 m. covered by 1 cm. galvanized wire netting. These were pressed firmly down over the spots where the seeds were buried, and fastened rigidly to the ground by stakes nailed to the sides. Normal growth of ground vegetation was not interrupted. The seeds were buried in pockets about 5 cm. beneath the surface, the duff being lifted with a knife for the purpose. About 500 seeds were placed in each pocket, and marked with a small stake. About 20 lots of seed were accommodated in each cage. The seed used was collected and extracted in the fall of 1925. Wings remained attached to the seeds. The cages were established in three different forest types in the fall of 1925. In 1926 small copper gauze boxes containing a known number of seeds mixed with the duff were buried. With these it was possible to recover every seed buried. Whenever it was possible to visit the stations, temperatures in the duff were read from current thermometers permanently hung in wooden tubes.

TABLE III

GERMINATION OF RED SPRUCE SEED STORED IN THE DUFF, 100 SEEDS PER SAMPLE. TESTS MADE ON THE JACOBSEN GERMINATOR AT 24° C.

SAMPLE NO.	CHARACTER OF DUFF WHERE BURIED	TIME BURIED	PERCENTAGE GERMINATED AFTER			REMAINING SOUND SEED	GERMINATION CAPACITY
			30 DAYS	60 DAYS	90 DAYS		
		months	%	%	%	%	%
33	Spruce raw humus	2.5	26	26	26	2	28
37	" " "	3	0	0	0	0	0
34	" " "	5.5	0	0	0	0	0
7	" " "	7	32	42	44	0	44
8	Beech-maple leaves	6.5	0	0	0	0	0
19	" " "	11	0	0	0	0	0
13	Balsam-birch-aspen litter	11.5	0	0	0	0	0
15	Spruce raw humus	11.5	2	3	3	0	3
16	" " "	11.5	0	0	0	1	1
18	Balsam-birch aspen litter	11.5	0	0	0	2	2
35	Spruce raw humus	12	0	0	0	0	0
36	" " "	12	0	0	0	0	0
28	Balsam-birch-aspen litter	13	16	16	16	5	21
29	Balsam-birch-aspen litter	13	28	28	28	2	30
21	Spruce raw humus	23	0	0	0	2	2
22	Balsam-birch-aspen litter	23	0	0	0	46	46
23	Beech-maple leaves	23	0	0	0	32	32
39	Balsam-birch-aspen litter	24	8	8	8	8	16
25	Spruce raw humus	33	0	0	0	0	0
20	" " "	33	0	0	0	1	1
26	Beech-maple leaves	33	0	0	0	9	9
27	" " "	33	0	0	0	21	21
	Control samples of same lot of seed, stored in laboratory, and germinated at same time as above						
1	Dry, in corked bottles	0	58	60	60	9	69
3	Dry, in corked bottles	3	43	48	48	7	55
11	Dry, in corked bottles	6.5	12	48	53	2	55
14	Dry, in corked bottles	11.5	43	44	44	1	45
17	Dry, in corked bottles	11.5	28	37	37	0	37
24	Dry, in corked bottles	23	63	63	63	23	86

Better success attended these experiments. Samples removed from the duff were tested in the JACOBSEN germinator simultaneously with controls of the same lot of seed kept in air-tight containers in the laboratory. The results are shown in table III. The latter lot of seed lost little germinative energy during 25 months, while that stored in the duff decreased 50 per cent. in eight months and after 11.5 months gave only 1.7 per cent. as great germination as the control. In one case 8 per cent. germination was obtained after two years, but this was exceptional. In many cases seeds were found which had germinated in the duff. Where moisture was adequate, conditions were favorable for germination throughout the growing season, since the temperature was sufficiently high (table V), as will be shown later. Seeds which had after-ripened by storage during the winter were able to germinate at still lower temperatures. This tendency to germinate as soon as conditions became favorable probably explains why so few seeds remained dormant over summer. HAIG (**20**) has recently reported the same phenomenon in western conifers.

SUMMARY OF FIELD EXPERIMENTS

From seed plot trials the importance of moisture conservation in the surface soil and of moisture availability was apparent. Germination was most successful on sand or mineral soil and under cover of spruce branches or needles. Similar results were observed on extensive trials of direct seeding, and on plots depending on natural seeding. The removal of root competition by trenching was followed by more abundant germination. Some spruce seeds remained viable in the duff about one year, but had deteriorated greatly in germinative capacity by that time.

II. LABORATORY EXPERIMENTS

1. AVERAGE COURSE OF GERMINATION.—Seventy-six lots of red spruce seed tested during the years 1927–30 gave an average germination of 77 per cent. in 30 days for fresh seed and 62 per cent. in 30 days for 3-year-old seed. Tests were made in the JACOBSEN apparatus at a constant temperature of 24° C. Figure 4 shows smoothed curves of the normal course of germination after varying periods of dry storage in corked bottles at room temperature. Fresh seed germinated more promptly and gave higher energy values at 30 days than seed which had been stored longer. In the stored seed, that 2–3 years old germinated slightly more promptly than that 1–2 years old. Final values were higher for the latter, however. The germinative energy in 30 days was found to decrease about 10 per cent. per year for the first three years. With increasing age there ensued a progressive decrease in germinative energy, as well as in the final germination percentage and germinative capacity. Frequently the embryo and endosperm became

TABLE IV

AVERAGE VIABILITY OF SEED AFTER STORAGE IN AIR-TIGHT CONTAINERS AT ROOM TEMPERATURE
AVERAGE COURSE OF GERMINATION FOR 1927–1930,
JACOBSEN GERMINATOR. TEMPERATURE 24° C. CONSTANT

	COURSE OF GERMINATION AVERAGE PER CENT. GERMINATED AFTER						GERMINATIVE ENERGY IN 30 DAYS			REMAINING SOUND SEED AV.	GERMINATIVE CAPACITY AV.	BASIS NO. OF TESTS OF 100 SEEDS EACH
	5 DAYS	10 DAYS	15 DAYS	20 DAYS	25 DAYS	30 DAYS	HIGH	AV.	LOW			
	%	%	%	%	%	%	%	%	%	%	%	
Seed stored less than 1 year	13	66.5	76.0	76.7	77.2	77.2	87	77.2	66	7.0	84.2	4
Seed stored 1–2 years	0.4	40.7	59.2	65.8	67.0	68.0	91	68.0	27	7.8	75.8	47
Seed stored 2–3 years	6.3	48.7	54.4	58.6	60.3	62.0	88	62.0	30	9.2	71.2	25

shriveled and nearly disappeared in old seed, giving rise to an apparent increase in the percentage of empty seed. By suitable recleaning, these blind seeds can be eliminated, thus restoring to a lot of seed much of its original germinative value.

2. GERMINATION AT DIFFERENT CONSTANT TEMPERATURES.—Temperature affects seed in many ways. Summer heat affects the ripening of the seed, the heat (through its drying effect) influencing extraction from the cone, whether by natural or artificial means. Temperature is important while the seed is in dry storage or in the process of after-ripening in the soil, and finally, it is frequently a limiting factor in germination.

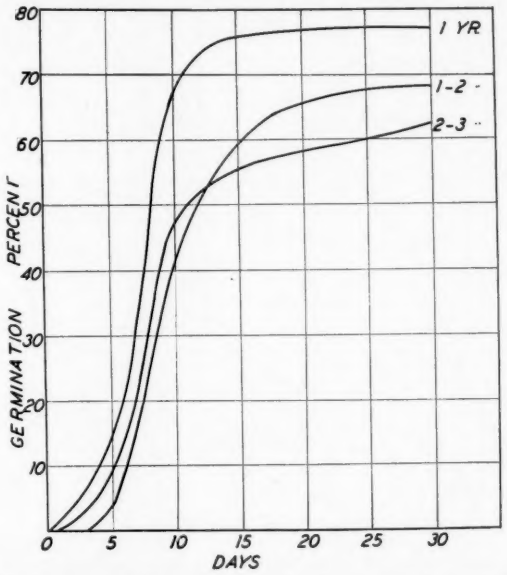

FIG. 4. Average curves of germination of red spruce seed grouped by duration of storage. JACOBSEN germinator at 24° C. constant. Basis one year, 800 seeds; 1–2 years, 9400 seeds; 2–3 years, 5000 seeds.

The following experiments were made to determine the temperature requirements for germination of red spruce. They were begun in March, 1930, and extended over six months.

Seeds of the 1928 crop were used, 800 for each temperature at which tests were made. They were counted out from the air-dry seed and set to germinate without treatment, except in a few cases. Containers for the seeds were prepared as follows: Petri dishes 10 cm. in diameter were filled to a depth of 1 cm. with pure quartz sand. These were covered with filter

papers fitting the dishes exactly. The dishes were then saturated with distilled water and the excess drained off by tipping the dish. Then 100 seeds were laid on the filter paper and the dishes covered. Moisture was renewed at the time of counting the sprouted seeds.

The seeds were germinated in a specially constructed series of Freas chambers at the following temperatures: 10°, 20°, 24°, 28°, 32°, and 38° C. The petri dishes were placed in stacks of four each, and at each counting the positions of the dishes in the stack were changed. The tests were examined daily at the same hour, and the sprouted seeds removed, usually at two-day intervals. The seeds were kept in darkness except for 2–3 minutes at the time of each examination. Uniform temperatures were maintained

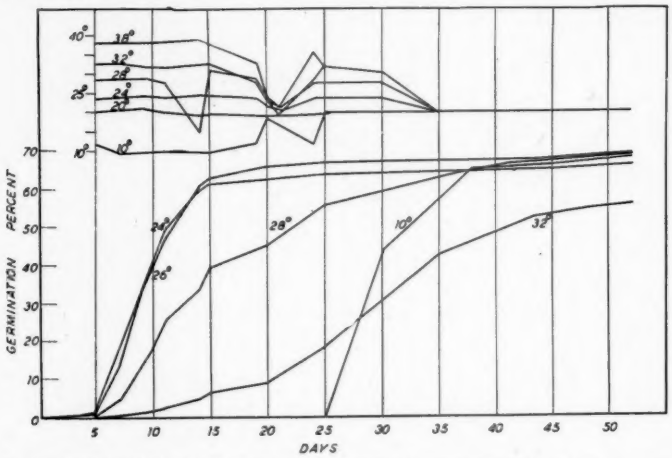

FIG. 5. Germination of red spruce at different temperatures, first series. Basis 800 seeds each curve.

except for the following fluctuations: On the 11th day the 28° chamber suffered a sharp drop in temperature, and after the 20th day all chambers were brought to 20° for four days. At the expiration of this time the original temperatures were resumed and maintained until the 30th day. On the 25th day, no germination having occurred in the samples stored at 10°, the samples were removed from the ovens to a cellar where they remained at approximately 20° C. in diffuse light until the tests were discontinued. The fluctuations in temperature are shown graphically in figure 5.

In May, 1930, a second series of tests was set up in the same ovens, using samples from the same lot of seed. The same germinating medium was used as in the first series, but in this case the petri dishes, complete

with sand, filter paper, and water, were autoclaved for 15 minutes at 20 lb. steam pressure and cooled to room temperature before inserting the seed. In this experiment all samples were examined, and sprouted seeds removed and recorded daily at the same hour. Temperatures in the chambers were read to 0.1° C. at each examination. During this second series two changes in the uniformity of the temperature occurred, one for six hours on the fourth day, and again for an equal period on the eighth day. In addition, the temperature in the 26° chamber rose to 32° C. for a period of unknown length on the thirteenth day. The temperatures maintained (except as just noted) were 10°, 15°, 18°, 20°, 24°, and 26° C.

The summarized results are shown in table V and figures 5–7. In the first series the samples at temperatures of 20° and 24° were the only ones displaying a normal course of germination. No germination whatever occurred at 10° or at 38° C. At 28° C. the inhibiting effect of high temperature was already very marked, and satisfactory germination began only after the drop in temperature on the eleventh day already mentioned. When the ovens were shut down and the temperature in this compartment fell, germination was completed normally. The same was also true of the samples at 32° C. The seeds exposed to 38° C. were probably killed after a short period, since a subsequent drop in temperature did not induce germination. In the case of the low temperatures, 10° C. was below the minimum for germination. In neither of the series did germination occur at 10° C. during the period of the tests (20–30 days). In the second series, four samples which had been after-ripened in moist peat at 5°–7° for two months were incubated at 10° C., but no germinations occurred. Upon completion of the tests, these samples, as well as the others from the 10° C. oven, were placed in a refrigerator and kept continuously at 5°–7° C. for 133 days. During this time 1–2 per cent. germinated, indicating that, as a result of prolonged after-ripening, the minimum temperature for germination had become lower. These samples were then placed in the ground under a spruce stand in New Hampshire where the temperature varied from 0° to 5° C. They were allowed to remain there one month, during which 4 per cent. germinated. Then extreme weather necessitated their removal to a cool cellar. During the next month in the cellar at approximately 8° C. about 20 per cent. more germinated. The tests were then discontinued.

In October, 1930, experiments were made to determine whether seeds would germinate out-of-doors under the prevailing temperatures. Owing to the severe drought prevalent at the time, it was arranged to keep the seed-bed uniformly moist, while otherwise simulating natural conditions as closely as possible. The following apparatus was used: copper cylinders 10 cm. in diameter and 15 cm. high were constructed, having bottoms of very fine

TABLE V

SUMMARY OF GERMINATION OF RED SPRUCE AT DIFFERENT CONSTANT TEMPERATURES*

TEMPERATURE	SERIES	COURSE OF GERMINATION — DAYS																						
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	35	38	43	48	52
°C.		%																						
10	I																	0.1	43.6		65.0			68.0
	II	(1.7% after 133 days, 5.5% after 160 days, 26.2% after 190 days)																						
15	II					0.8	2		5.4	11.4	15.9	24.9	26.9	30.8	32.5	33.6	36.6	38.6						
18	II			1.2	7.1	17.6	28.4	38.6	48.1	55.4	60.0	61.7	64.1	65.7	66.4	68.1	68.9							
20	I			12.5		31.9		45.9			60.0	62.2					65.0	66.1	66.4	66.6		67.2	68.1	68.9
	II		0.4	13.0	27.6	37.5	45.6	53.2	56.4	59.1	61.8	62.6	63.5	63.7	64.1	65.0	65.2							
24	I	0.7		16.9		32.6		48.6			59.2	61.2					62.7	63.7	64.0	64.2		65.1	65.2	65.9
	II	2.8	6.5	24.5	36.2	44.2	52.4	57.4	60.5	62.7	64.0	64.6	65.0	65.2	66.0	66.2	67.0							
26	II	3.0	11.5	28.5	38.4	50.2	58.4	61.5	62.8	65.0	66.0	66.8	67.0	67.4	67.9	68.5	69.1							
28	I	0.4		4.6		12.7		25.1			33.2	39.1					44.5	55.7	59.2	62.7		66.1	68.1	68.5
32	I			0.4		1.1		2.2			4.7	6.6				8.7	9.0	18.2	30.4	42.5		52.0	54.5	56.2
38	I	No germination																						

* Basis 800 seeds each temperature and series; total 9600 seed.

TABLE V—(*Continued*)*

Temperature	Series	Remaining seed			Germination values		Variation between samples	
		Good	Bad	Empty	Real germination in 20 days	Real germinative capacity	Average deviation	Standard deviation
°C.								
10	I			8.5	42.7	83.5	5.5	6.9
	II			3.1	70.8	87.2	4.4	4.7
15	II	43.7	8.0				3.2	4.3
18	II	17.4	9.5	7.2	71.2	87.4	5.7	6.8
20	I	15.0					7.2	9.8
	II	12.0	7.7	5.0	71.8	90.5	2.4	3.2
24	I			2.4	72.5	88.4	2.8	3.6
	II	17.5	8.6				3.2	4.7
26	II	15.2	10.8				1.7	2.6
28	I							
32	I							
38	I							

* Basis 800 seeds each temperature and series; total 9600 seed.

(80-mesh) copper gauze. They were mounted on 1.5-cm. legs. These cylinders were filled to within 2 cm. of their tops with fine sand, previously cleaned by washing with 20 per cent. HCl and distilled water. The cylinders were then placed in an enameled pan filled with water to a point 1 cm. above their wire bottoms. This level was subsequently maintained automatically by a Mariotte flask rigidly attached to the pan. The remainder of the water surface in the pan was covered with glass strips held in place with adhesive plaster and heavy paraffined cardboard to prevent evaporation and to exclude dirt. The whole was then given a coat of waterproof lacquer. The apparatus was buried in the ground so that only the tops of the cylinders protruded. The sand surface within the cylinders was adjusted level with the surface of the ground (fig. 8). Two such complete units were established, containing three cylinders each, one in an opening and one under dense shade in a 20–40 year-old stand of old field spruce near Berlin, N. H. The ground surface in the open was covered with a heavy sod and spruce seedlings were abundant. Under the stand there was no ground flora, but a thick mat of spruce needles covered the surface. One hundred seeds of the 1928 crop were sown in each cylinder and gently pressed into contact with the sand. Maximum and minimum thermometers with bent stems for measuring surface soil temperatures (**31**) were kept inserted in the sand surface and read daily. When temperatures continuously below freezing ensued in mid-November, no germination had yet occurred in any of the cylinders, and both units were removed to the laboratory. Here germination began promptly and continued for nearly a month from seed which had become deeply buried. Owing probably to the poor quality of the 1930 crop, some seeds collected from trees on the site of the experiment and sown along with the standard seed failed to germinate. Probably the experiment should have been started earlier in order to encounter conditions suitable for fall germination. Seed shed early in September may thus in some years meet with temperatures favorable for germination. Periods of warm weather also occur occasionally in October. From several years' observation, however, only exceptionally have fall-germinated red spruce seeds been seen.

Summary of effect of temperature

The effective range of temperature within which germination occurs normally was found from the preceding experiments to be approximately 15°–30° C. The optimum temperature apparently lay between 24° and 28° C. For after-ripened seed the minimum was lower. Imbibed seeds exposed to temperatures of 32° C. and higher seemed to incur permanent injury. On the other hand, incubation at temperatures below the effective range resulted in very rapid germination when the temperature was raised to a point within the optimum range.

3. RATE OF MOISTURE ABSORPTION.—Red spruce seeds belong to the group with moderately high germinative energy. They germinate in a few days after the conditions become favorable. PETTIS (**40**) gives the time necessary for red spruce seeds to germinate in the nursery as 12 days. The shortest time between placing dry seeds in the germinating medium and the starting of germination observed by the writer was four days; seven days is perhaps the average. Seeds buried in soil do not appear above ground until

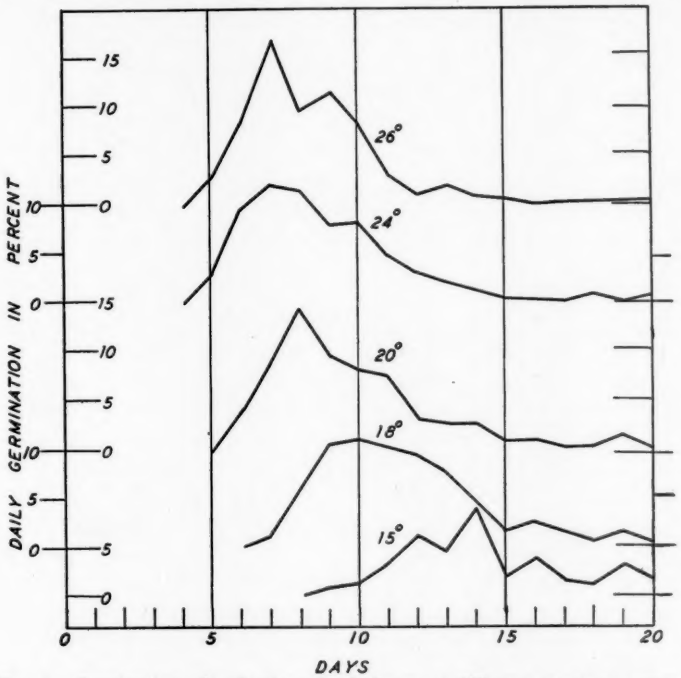

FIG. 6. Germination of red spruce in darkness at different constant temperatures (° C.), second series. No germination occurred at 10°. Basis 800 seeds each curve.

considerably later. The peak is usually reached by the 12th day, and germination is nearly over by the 20th day in germinators. A few seeds continue to germinate for 30–50 days (fig. 5). The beginning of germination is closely correlated with the rate of water absorption or imbibition. Seed coats of red spruce seeds imbibe water readily, as shown by the following experiment.

Two samples of 100 seeds each of the 1928 crop, containing 7 per cent. moisture based on dry weight, were placed in individual JACOBSEN germinators (**2**) with a water level 10 cm. below the plate. The seeds were weighed

on an analytical balance before being placed in the germinators, and at intervals until germination started. In weighing, the seeds were brushed carefully on to a dry filter paper which had been balanced on the pan, and the weighing made as rapidly as possible, to avoid loss by evaporation. A fresh paper was used for each sample. After the final weighing, the seeds

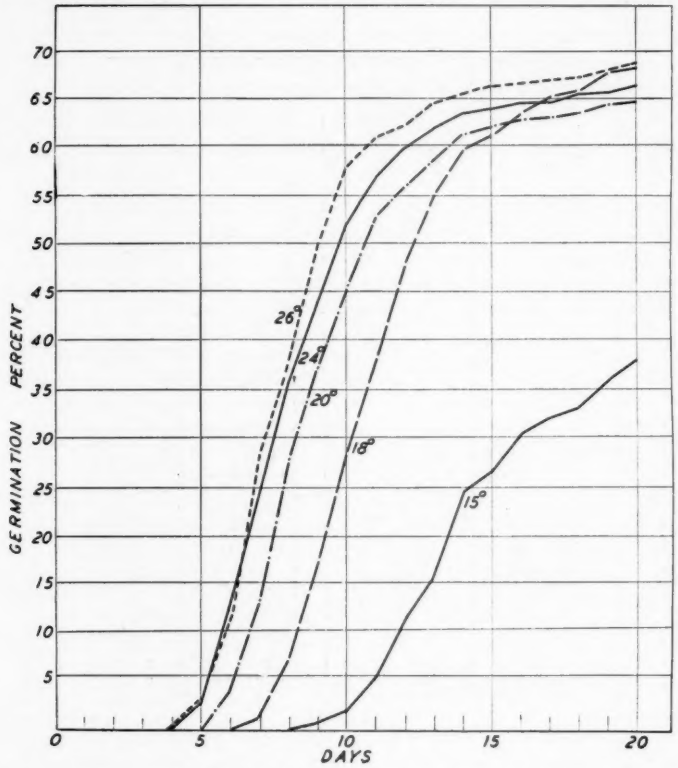

FIG. 7. Daily germination of red spruce seed at different constant temperatures (° C.), second series. Basis 800 seeds each curve.

were oven-dried at 105° C. for 48 hours and the dry weight determined. The moisture percentages are illustrated in figure 9. The rapid initial absorption demonstrated that the seed coats were readily permeable.

RATE OF WATER ABSORPTION AS RELATED TO VOLUME.—Upon imbibition, the volume of the seed plus that of the imbibed water increased. This is not true of all seeds. The behavior of red spruce seed was demonstrated by the following experiment:

A small bulb was blown in one end of a glass tube, sealed off and filled with air-dry seeds. The tube and bulb were filled with boiled distilled water, care being taken to eliminate air bubbles. The tube was then attached to a graduated manometer tube filled with mercury, similar to the apparatus described by DU SABLON (**45**). The height of the mercury column was read at intervals for two weeks, the apparatus standing on the laboratory desk in

FIG. 8. Apparatus in place for studying fall germination.

diffuse light at about 22° C. A continuous rise occurred, increasing to a maximum at seven days, and then remaining constant. The trend was as follows:

INCREASE IN VOLUME AFTER:					
4 hrs.	6 hrs.	28 hrs.	2 days	7 days	12 days
0.04 ml.	0.05 ml.	1.90 ml.	1.92 ml.	2.70 ml.	2.70 ml.

The rate of increase in both weight and volume due to imbibition of water will be seen to be closely correlated with the time elapsing before germina-

tion commenced. OSTERHOUT (**38**) found the rate of water absorption much greater at high temperatures than at low ones, and it seems probable that the hastening of germination observed at higher temperatures may be explained by the more rapid intake of water and the speeding of chemical reactions according to the van't Hoff law.

4. GERMINATION IN LIGHT AND DARKNESS.—The first experiments on the effect of light upon the germination of red spruce seeds were made in March, 1930, in the greenhouse of the Marsh Botanical Garden. Eight samples of 100 seeds each of the 1928 crop were placed in a dark chamber and eight in the light chamber. The seeds were germinated in petri dishes as in the

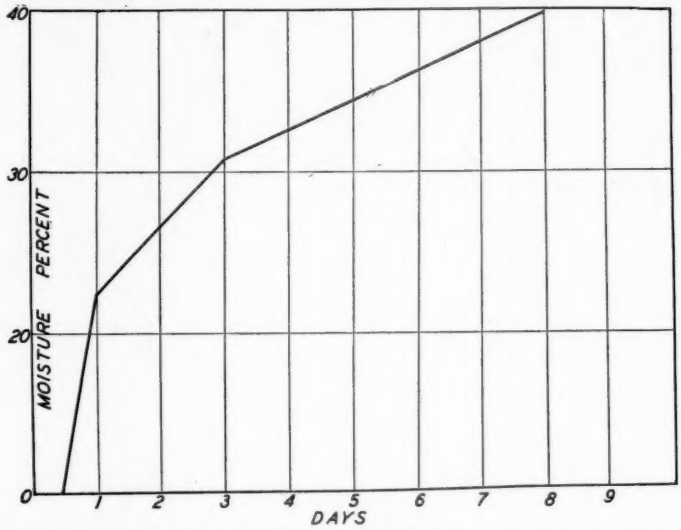

FIG. 9. Course of moisture absorption by red spruce seed prior to germination on JACOBSEN germinator.

experiments with temperature, and the apparatus for securing darkness was the same chamber as was used by DILLER (**16**) and HUTCHINGS (**22**) in their studies on the effect of light and darkness on germination. Sprouted seeds were counted and removed at intervals of five days. Final cutting tests were made on all seeds which did not germinate within 30 days. Temperatures in the chambers read with current thermometers at the time of counting the sprouted seeds varied from 21° to 24° C., which fell within the effective optimum range for normal germination (*q.v.* above).

The results are shown in table VI and in figure 10. After 10 days about 10 per cent. higher germination was recorded in darkness than in light;

hypocotyls were longer in the dark, possibly because of the absence of the inhibiting effect of light. After 15 days the difference between the light and dark samples had become less, and at 20 days the number germinated in light exceeded that in darkness. At 30 days the average germinations of the two series varied by but 1.5 per cent., and real germinations[2] showed a mean

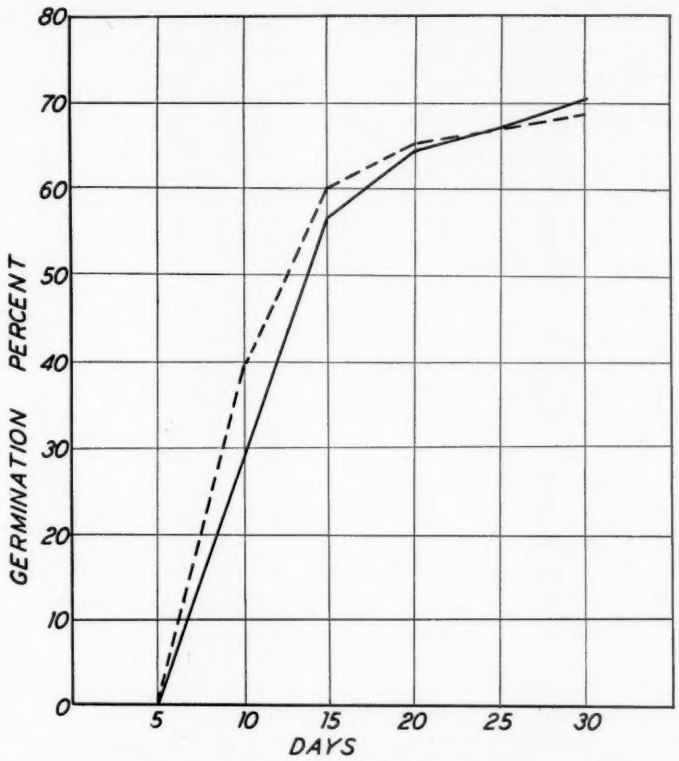

FIG. 10. Germination of red spruce in light and darkness. Dotted line indicates darkness. Basis 800 seeds each curve.

difference of but 0.8 per cent. A computation of average and standard deviations from the mean indicated that the two series exhibited essential uniformity, and no significant difference in behavior. Both standard deviations and probable error were greater in magnitude than the difference in germina-

[2] "Real germination" is based on the percentage of sound seeds germinating, and thus differs from the apparent germination of the entire sample, when many empty seeds are present.

TABLE VI

AVERAGE GERMINATION OF RED SPRUCE SEED IN LIGHT AND DARKNESS

YEAR COLLECTED	DATES TESTED	GERMINATION TEMPERATURE	APPARENT GERMINATION IN 30 DAYS		REAL GERMINATION (PER CENT. OF FILLED SEED GERMINATED IN 30 DAYS)		BASIS (NO. OF SEED)
			LIGHT	DARKNESS	LIGHT	DARKNESS	
		°C.	%	%	%	%	
1928	Mar. 1930	24	70.0	68.5	75.2	76.0	1,600
1929	Nov. 1930	"	80.5	69.2	82.0	70.0	800
"	Dec. 1930	"	77.2	69.0	79.4	70.5	800
"	Jan. 1931	"	71.2	62.2	73.0	63.2	800
"	Feb. 1931	"	80.5	60.7	81.0	62.2	800
"	Mar. 1931	"	69.0	51.5	74.3	56.1	800
"	(Av. of 20 monthly tests		70.34	54.17	.....	.....	16,000

tive energy between the light and dark series. The experimental error was thus greater than the observed difference between the two conditions.

Beginning in November, 1930, a series of tests was started, primarily to study periodicity of germination. Unfortunately, change of residence necessitated the abandonment of these tests in June, 1932, after 20 months. They give, however, abundant data on germination in light and in darkness. Four samples were set up in light and four in darkness on the JACOBSEN germinator at 24° C. on the first of each month. Darkness was secured by coating the inside of the glass bell-jars with black enamel and India ink, while the outer surfaces were painted with white enamel to prevent absorption of heat. Thermometers were inserted in both dark and light samples and read daily. The temperatures were practically identical under both conditions, and, since temperature was controlled thermostatically, no fluctuations occurred. The results are given in table VI. They show from 10 to 30 per cent. greater germination in 30 days in light than in darkness, and suggest that light may be definitely beneficial in hastening germination, although by no means essential.

After-ripening.—Since red spruce sheds much of its seed in the autumn, which then remains on the wet ground under the snow, or in contact with melting snow until late spring before germinating, it might be supposed that cool moist storage of the seed would be the natural condition, to which the seed had become accustomed for centuries, and that stratification under such conditions would have the effect of hastening germination. To test this the following experiments were made:

TABLE VII

AVERAGE GERMINATION OF 1½ YR.-OLD RED SPRUCE SEED WITH AND WITHOUT AFTER-RIPENING

DATE COLLECTED	DATE TESTED	AFTER RIPENING TEMPERATURE	PERIOD OF AFTER RIPENING	GERMINATIVE ENERGY IN 30 DAYS		GERMINATION TEMPERATURE		BASIS NO. OF SEED
				GERMINATOR	SOIL	GERMINATOR	SOIL	
		°C.	*months*	*%*	*%*	*°C.*	*°C.*	
October 1928	February–March 1930	10	2	69	71	15–20	10–35	300
		10	2.5	83.5	77	15–20	10–35	700
		Controls		79.5	47.5	.....	.....	700
October 1929	February–March 1931	7	1	73.1	.....	26	.....	800
		Control	...	67.1	.....	26	.....	800
		7	2	70.6	.....	26	.....	800
		Control	...	50.1	.....	26	.....	800

Seed of the 1928 lot was stratified in moist peat at 8°–10° C. for approximately two months. Part of the seed was then removed and germinated along with similar samples of untreated seed, in individual JACOBSEN germinators. The germination temperature varied from 15° at night to 20°–25° C. by day. Part of the stratified seed was allowed to dry for two weeks. The remainder was dug up and samples of the freshly dug seed, seed which had been dried two weeks after stratification, and unstratified controls were germinated in soil flats in the greenhouse. The flats were prepared as described by TOUMEY and STEVENS (**58**). The sprouted seeds were counted and removed at 5-day intervals.

The results are given in table VII and shown graphically in figures 11, 12. In every case moist storage at low temperatures resulted in an increase in germinative energy and capacity as well. The differences, however, between the courses of germination of stratified and unstratified seeds were in no case as great as found for some other conifers by other investigators (**44, 39, 59, 60, 5, 6** and others), or by the writer (**3**) for eastern hemlock (*Tsuga canadensis* (L.) Carrière.)

In December, 1930, another series of experiments on after-ripening was started. Seed of the 1928 crop was used. One hundred seeds were placed on a layer of moist granulated peat 1 cm. thick in each petri dish, and covered with a thin layer of powdered peat. The dishes were then stored in a cold cellar at 6°–8° C. At the end of one month and of two months eight samples each were removed and placed at 24° C. to germinate along with eight similar samples of dry seed, freshly placed in peat as controls. Every five days the dishes were examined for sprouted seed and the peat worked over with tweezers to insure proper aeration. Water was added if necessary. The summarized results are shown in table VII and figures 11, 12. No significant difference in germination was observed between the two sets of samples. What little hastening of germination occurred in the stratified samples can be explained by the fact that these seeds were already fully imbibed as compared with the dry seeds.

SUMMARY OF RESULTS OF AFTER-RIPENING EXPERIMENTS

Field observations by a number of workers, as well as laboratory tests, show that seeds of several species of spruce cannot be classed as requiring prolonged after-ripening before becoming capable of normal germination. The present experiments confirm this conclusion; germination was hastened, but to a slight degree only.

5. GERMINATION UNDER AERATING WATER.—It is generally believed that excessively moist seed-beds are unfavorable for germination, the poor results obtained under such conditions being usually attributed to lack of

aeration. When seeds swell in water their respiration rate increases, and if sufficient oxygen is not at hand death ensues. TOUMEY and DURLAND (**54**) found that seed of upland species soaked over five days in water at greenhouse temperature gave very low germination, if it germinated at all.

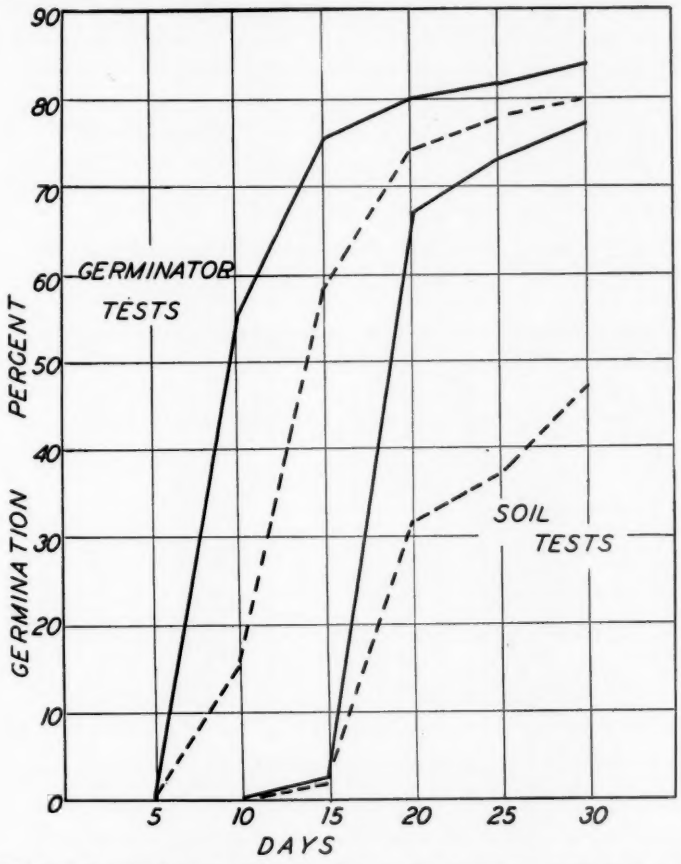

FIG. 11. Germination of after-ripened seed of red spruce. Solid lines represent seed stratified 75 days at 10° C. Dashed lines, controls. Basis 1700 seeds.

With white spruce the germination fell off constantly with longer soaking, until no germination was secured after 30 days. Red spruce was not tested. In the present experiments the seeds were kept totally immersed and germination in aerated and unaerated water compared.

Two hundred seeds of the 1928 crop were attached to both sides (100 on a side) of a glass strip 2 cm. wide, by coating the strip with shellac and

pressing the seeds into it. The glass strip was inserted into a cork fitting the neck of a 2-liter bottle and suspended vertically in the bottle. Two such bottles were set up and filled completely with water which had been boiled to expel all dissolved air and then cooled. One bottle was arranged to be constantly aerated by blowing a stream of air through it from a compressed air tap, leading the air to the bottom of the bottle through a glass

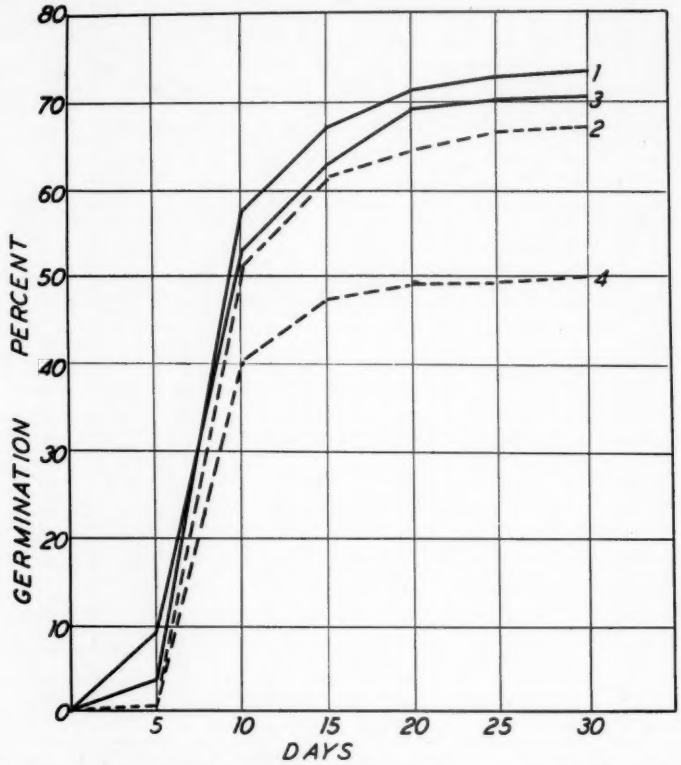

FIG. 12. Germination of after-ripened seed of red spruce: (1) Stratified one month at 7° C. (2) Control. (3) Stratified two months at 7° C. (4) Control (basis 800 seeds each curve.)

tube. The other bottle was not aerated. The bottles were kept at room temperature, averaging about 23° C.

After nine days some seeds in the aerated bottle began to germinate, and 13 days after the start of the experiment, aeration of this bottle was discontinued and the control bottle attached to the air tap. Up to this time

no germination had occurred in the control bottle. Six days after aeration of the control bottle had begun, some seeds were noticeably swollen, and after 11 days about 10 per cent. had germinated. This showed that even seeds soaked 13 days in the absence of oxygen retained their vitality.

Final counts from both bottles were as follows (basis of 200 seeds):

(1) Dry seed placed in water and aerated 13 days ..... 57 per cent.
(2) Dry seed placed in water without aeration ..... 0 per cent.
(2) after subsequent aeration for 13 days ..... 28 per cent.

These experiments suggest that red spruce seed is capable of enduring considerable soaking and storage under anaerobic conditions without losing its viability. Presumably submergence at lower temperatures than those used in these experiments, and such as would occur in nature, would have an even less deleterious effect. Submergence at higher temperatures would shorten the period the seeds would remain viable, as shown in TOUMEY and DURLAND'S experiments (**54**).

TABLE VIII

GERMINATION OF RED SPRUCE SEED IN SOLUTIONS OF DIFFERENT pH
GERMINATIVE ENERGY AT 21 DAYS. EACH FIGURE IS THE AVERAGE OF 200 SEEDS

pH	Series and date							
	I Feb. '28	II*—III May '28	IV Oct. '28	V Dec. '28	VI Mar. '29	VII Apr. '29	VIII†—IX† Sept. '29	
	%	%	%	%	%	%	%	%
1.05					0	0		
2.05					**70**	44		
2.5					67	64		
3.0		**60**		51	54	**56**‡ **59**‡		
3.5		49		44	58	56		
4.0		47		**56**	51	73.5		**71**
4.5		49		56	molded: unreliable*		75	46
5.0		40		34	"	52	77	54.5
5.5	**88**	52	**26**	26	43	48		
6.0	83	41	**26**	44	57	**47**‡ **19**‡		
6.5	76	54	17	34	31	30		
7.0	19	33	6	41	33	25	**77.5**	60.5
7.5								
8.0	17	35			1.5	41	70.5	65
8.5							51.5	3.5
Controls (distilled water)	88	51	35	24	66	52	77.5	73.5

* Results unreliable owing to excessive mold; results not given.

† Figures give germination at 19 days for series VIII and 17 days for series IX respectively.

‡ Two overlapping solutions of same pH.

Figures in bold face type indicate best germinations for each series of tests.

GERMINATION IN DIFFERENT HYDROGEN ION CONCENTRATIONS.—Several students of spruce reproduction have attached importance to the acidity of the seed-bed as a factor controlling the establishment of spruce. Field observations showed that seedlings grew in acid habitats. Table IX gives

TABLE IX

REACTION OF SOILS IN WHICH SPRUCE SEEDLINGS WERE GROWING

CHARACTER OF HUMUS	pH VALUE	MONTH
Northern hardwood leaf mold	6.5	May
Birch poplar leaf mold	6.5	May
Mineral soil under northern hardwoods	6.8	May
Red spruce humus (duff)	6.5	November
Raw humus under young spruce stand	4.8	April
Dry undecomposed spruce needles	4.3	October

the average pH values of soil clinging to roots of spruce seedlings a few months to a year old on various sites. They were averaged from a large number of determinations made near Cupsuptic Lake, Maine, in 1924 and 1925, using a LaMotte indicator field set.

An optimum degree of acidity for the germination of Norway spruce (*Picea excelsa* Link.) has been noted by AALTONEN (**1**) and SCHMIDT (**48**). The latter germinated seed in buffered solutions, and pointed out the danger of the results being influenced by the character of the solution unless overlapping tests were made with different salts to secure the desired pH range. He found the use of a single acid, such as sulphuric acid, unsuitable owing to the instability of an unbuffered solution.

Laboratory experiments were begun in February, 1928, and extended over 20 months. All-glass individual JACOBSEN germinators were used. Special care was taken in the cleaning of the glassware. It was soaked in acid bichromate cleaning fluid, rinsed, boiled in soap suds, rinsed in distilled water, and dried. The wicks were of unused pure cotton yarn. They were boiled in distilled water, and then each soaked in its corresponding solution before being put in place. High grade filter papers were placed on the wicks. Eight hundred-ml. pyrex beakers were used as reservoirs, filled to within 7.5 cm. in the first series, and to 10 cm. of the top in later experiments. After setting up the apparatus, the beakers were placed in the water bath of the large JACOBSEN germinator (fig. 2) and maintained at a constant temperature of 24° C. Two samples of 100 seeds each were set up for each different pH, and for each different solution. The same lot of seed of the 1927 crop was used in all tests. In series VI and VII the seed was counted with a special suction counter described by BROWN, TOOLE, and GOSS (**10**) and lent by courtesy of the Seed Laboratory, U. S. Depart-

ment of Agriculture. Wherever possible the ranges of different solutions were made to overlap 1.0 pH. Solutions were prepared varying by 0.5 pH from pH 1.0 to 8.5. The substances used for buffers included succinic acid, borax, sodium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, sodium chloride, citric acid, and glycocol. Before being used all solutions were diluted the same amount with distilled water, and their pH checked electrometrically by the calomel electrode method. At the end of the test the reactions of the filter papers were checked. A small quantity of liquid was sucked out of the filter paper with a dropper and its pH determined colorimetrically. In no case was any change detected, except in the last series, where the method of germination differed and the solutions were exposed to the air, and so could absorb CO_2. The experiments were repeated eight times. Results are presented in table VIII.

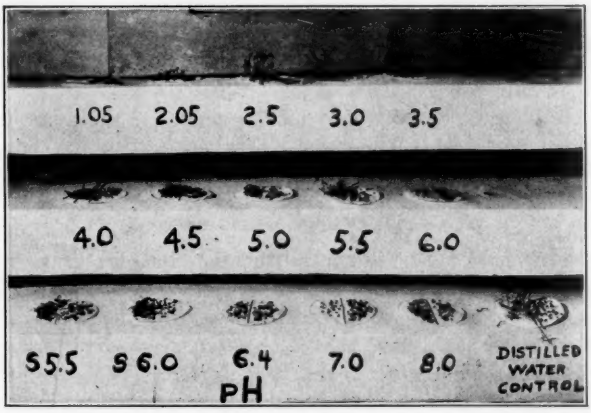

FIG. 13. Red spruce seedlings germinated in solutions of different pH.

In the first five series measurements were made of the lengths of tops and roots of seedlings in each sample. Twenty-five or more seedlings were drawn at random from each sample and measured on a millimeter scale. They were then weighed and the weight of 50 seedlings in grams computed for each sample.

In all the series, striking differences were observed in the germination and growth over different hydrogen-ion concentrations. These were apparent after the first week. The first seeds to germinate were invariably in the more acid samples in the range pH 3.0–5.5. Neutral and alkaline tests showed retardation in germination at the start, and after three weeks the seedlings had made much poorer growth than those on acid samples, or had died. Mold attack was most severe in the case of neutral and alka-

line samples. Solutions of salts in most cases seemed to have an unfavorable effect upon germination as compared with distilled water, but the effect of acidity could be noted in samples of the same solution with different pH values. In some cases better germination was secured in the buffered solutions than in the distilled water controls. The detailed tables showing courses of germination under the different conditions and data on weights of seedlings are omitted on account of lack of space. The appearance of the seedlings as shown in figure 13 gives a much better idea of the effect of the various degrees of acidity than would the numerical data.

Summary of results of germination in different hydrogen-ion concentrations

Germination was found to take place more rapidly and completely in the more acid solutions than in neutral and alkaline ones. In most cases alkaline solutions proved distinctly injurious. The range of tolerance on the acid side was very wide; good germination was obtained from pH 2.0 to 6.6. The superiority of highly acid substrata may have been due in some degree to the greater freedom from mold. A pronounced optimum pH could not be found. In general red spruce seeds germinated best between pH 3.5 and 5.5. Figure 14 presents some of the results graphically.

Discussion of results

The experiments with different incubation temperatures showed that germination occurred at a wide range of temperature. Thirty-eight degrees C. was found definitely too high, which agrees with the findings of Haasis (**19**). If the seeds could have been kept perfectly sterile it is possible that some might have germinated. Thirty-two degrees was above the optimum, which probably lies between 24° and 26° C. The differences in germination between any temperature from 18° to 28° C. were chiefly due to hastening germination in the early stages of incubation by faster water absorption and chemical changes in the seed. The end results after 20 days were nearly identical. Figs. 5–7 show clearly the influence of higher temperatures in hastening germination, in crowding the bulk of the germination into a shorter period, and in causing it to begin earlier. Apparently germination can occur a few degrees above freezing if the seed has been stored long enough previously at low temperatures. The lower the temperature, the more important becomes the time factor, and the period of germination is enormously extended at low temperatures, as observed by Haasis (**19**). No germination occurred at 10° C. after one month's trial.

The effectiveness of long incubation at low temperatures in lowering the temperature requirements has an important bearing on the after-ripening experiments. In these tests after-ripening was found to have little effect

on the rate of germination at relatively high temperatures, the optimum range for untreated seed. The capacity of the seed to germinate at low temperatures also helps to explain its failure to remain viable in the duff, assuming, of course, that moisture is adequate.

In spite of the more or less negative results of the first experiments on light and darkness, the later experiments seem to indicate that germination

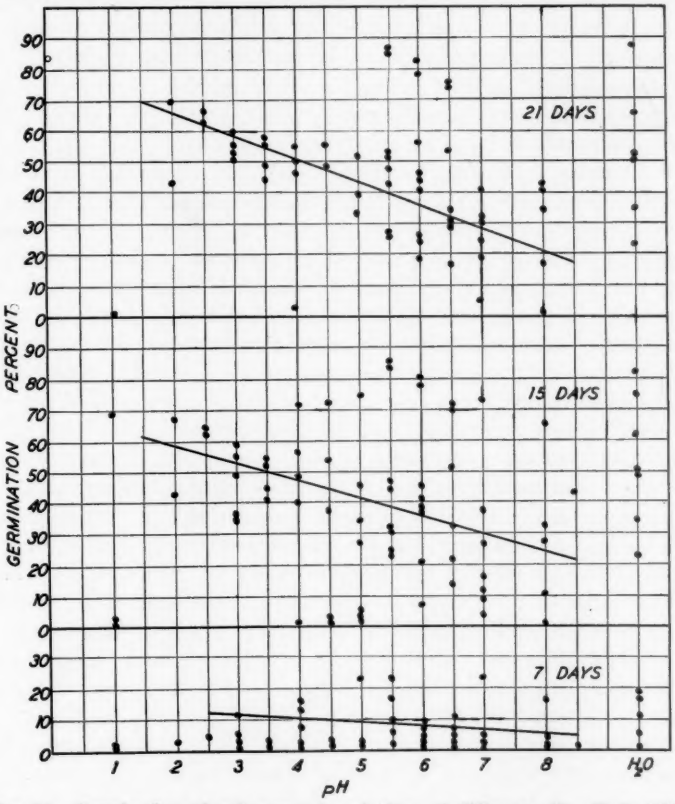

FIG. 14. Germination of red spruce in solutions of different pH. Basis 200 seeds each dot.

is hastened somewhat by light. In the first series the average germination after 30 days in light and darkness varied but little. In the 20 monthly tests carried out subsequently, dark germination exceeded light in but one case, and the excess of light over dark was generally 10 per cent., and once or twice about 30 per cent. Lack of light seems to retard the rate of germi-

nation, not to prevent it. Light probably acts as a stimulant to set in motion the chemical changes preliminary to germination.

The effect of the combined action of light and temperature, especially low temperature, was not investigated. With a species which germinates as readily as red spruce, it seems doubtful whether the influence of such factors would be profound. In most cases where the combined action of light and low temperature (or darkness and low temperature) has been found effective in hastening germination, the seed germinated with great tardiness under ordinary germinator conditions (KINZEL **25–29**).

The rôle of oxygen in the germination of red spruce seeds may be important when the seeds are imbedded under hardwood leaves or mulch. While starchy seeds, such as peas and beans, can be germinated anaerobically because of the large amount of oxygen present in their storage foods, fatty seeds like spruce require oxygen from outside in order to oxidize their fatty reserves for use in germination. However, the oxygen requirements of spruce seeds appear quite minimal. More detailed experiments would be necessary in order to determine quantitatively the limits under which germination would occur.

The promptness of germination in red spruce seeds seems to be due to two chief causes: (1) their physiological readiness and (2) their comparative ease of moisture absorption. Seeds which are not markedly dormant must germinate when external conditions are favorable; if not, the chemicosynthetic processes are so weakened that they often cannot repeat the awakening. The inability of the seed to lie dormant over occasional periods of warm weather after seed-fall results at times in the devastating autumn germination of red spruce alluded to by many writers.

Water is the most important single factor in germination within the favorable range of temperature. Water, besides causing hydrolysis of the reserves in the seed, is responsible for the more or less mechanical swelling and bursting of the seed coat.

The short period that spruce seed was found to remain dormant in the duff can be interpreted in this light: conditions even approaching the minimum for germination mobilize the ferments; if the stimuli are then removed, the seed is weakened by the expenditure of energy, and will respond less vigorously on the next occasion. It is even more probable that it will actually germinate and be unable to reach the surface, or survive adverse climatic conditions. In examining seeds removed from the duff, black fungous threads were often observed enveloping them. HARTLEY, MERRILL, and RHOADS (**21**) state that considerable quantities of seed are destroyed by ordinary saprophytic molds, and suggest that failure to germinate in humus and leaf mold might be due to the attack of molds and even to damping off fungi like *Corticium vagum* B. & C. or *Pythium debaryanum*

Hesse. That seeds are killed by fungi before the coats are split has recently been demonstrated by RATHBUN-GRAVATT (**43**).

The experiments on germination in different hydrogen ion concentrations, in spite of inconsistent results in many cases, confirm fairly well the conclusions of other investigators that an acid reaction is not unfavorable to spruce seed; in fact, indications are that acid conditions more or less favor germination. If the illustrations of the seedlings at the end of 21 days (fig. 13) are compared with the corresponding figures for germination, it will be observed that the effect of the acid is more evident from the appearance of the seedlings than from the germination statistics. This agrees with the findings of SALTER and MCILVAINE (**46**) and SCHMIDT (**48**). It would appear that the seeds can germinate well from near neutral to pH 2.0. No data were obtained on mortality following germination, but seedlings growing in alkaline solutions died soon after germination, or exhibited blackening of the roots. From the curves for the course of germination it will be seen that the lower pH values not only permitted a greater final germinative energy in the period, but apparently stimulated earlier germination. This may account for the greater percentage of seeds germinating before being attacked by mold. While the controls over distilled water usually gave larger and more healthy seedlings, higher germination percentages were often recorded for the strongly acid samples. A comparison of the development of red spruce seeds in different reactions under laboratory conditions with those under the different acidities found in the field confirms their tolerance of a wide range of pH values.

These studies of the germination requirements show that no very narrow limits control germination; all requirements are met under average conditions during the growing season. Moisture, then, must be the limiting factor for germination much of the time. Probably most viable spruce seeds germinate promptly in the spring, but few seedlings survive. Lack of reproduction is more likely due to unsuitable conditions for survival and establishment than failure to germinate, provided of course that the seed supply is adequate.

Summary

1. Red spruce seeds were capable of germinating promptly following maturity under a wide range of conditions.

2. In the field, germination and survival were best on mineral soil.

3. Germination and survival were favored by surface soil moisture.

4. Under the conditions of the experiments the seed did not retain its vitality long when stored in the duff. Most of the seed germinated in the duff during the first season. A small percentage remained viable for one year, and occasionally for two.

5. The average germinative energy of freshly harvested seed was found to be 66–87 per cent. in 30 days, with an average of 77 per cent. During air-tight dry storage it decreased about 10 per cent. per year for the first three years.

6. Germination of air-dry seed took place within a temperature range of 15°–32° C. Germination was incomplete below 20° and above 28° C. The optimum range appeared to be 24°–26° C.

7. After prolonged stratification at low temperatures, the minimum temperature for germination was lowered.

8. After-ripening at 7°–10° C. in a moist substratum hastened germination only slightly.

9. Light was found non-essential, but somewhat stimulating to germination.

10. Moisture was most important, especially at the beginning of germination. Moisture absorption was rapid, and began immediately.

11. Germination took place under water when aeration was provided.

12. Red spruce seeds tolerated a wide range of acidity, and germinated well between pH 2.0 and 7.0. Germination increased up to pH 2.0 and was poorest toward the neutral point and alkaline side. Best seedlings resulted between pH 5 and 6.

13. The speed of germination was greater in the more acid samples.

14. The most vigorous seedling growth was obtained in the distilled water controls, but there was no evidence that the buffered solutions used retarded germination.

15. Molding of the seed was less severe at higher acidities.

16. These experiments indicate that natural reproduction of red spruce seeds is dependent not so much on the requirements for germination as on the factors which determine survival of the seedlings after germination takes place.

This investigation was made possible by cooperation between the School of Forestry, Yale University, and the Brown Company, Portland, Maine. Field studies were begun at Cupsuptic Lake, Maine, in 1924 and were continued at Berlin, N. H., until June, 1932. Laboratory work has been carried on at the Forest Investigations Laboratory of the Brown Company Forestry Division. The writer is especially indebted to the late Dr. JAMES W. TOUMEY, Professor of Silviculture in the Yale School of Forestry, under whose direction the investigations were carried out, for many helpful suggestions in planning and carrying out the studies, and in the analysis of the data and preparation of the original manuscript. He desires also to express his sincere appreciation to Mr. W. R. BROWN, for permitting the publication of the parts of the investigation carried on by the writer while employed by the Brown Company. Thanks are also extended to Professor

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THE CAROLINE A. FOX RESEARCH AND DEMONSTRATION FOREST
STATE OF NEW HAMPSHIRE, FORESTRY DEPARTMENT
HILLSBORO, N. H.

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CARBOHYDRATE AND NITROGEN RELATIONS IN WHEAT PLANTS WITH REFERENCE TO TYPE OF GROWTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

ANNIE M. HURD-KARRER AND ALLAN D. DICKSON

(WITH FOUR FIGURES)

Introduction

The growth habit of the wheat plant is strikingly affected by relatively small changes in the environment. If the short days of winter are lengthened by a few hours of artificial illumination, the young plants of Turkey, a winter variety, grow erect instead of assuming the recumbent growth habit, which in winter sowings normally characterizes the early tillering stage (**28**). Also, if this variety is grown at temperatures above 20° C., culm elongation may be completely inhibited, resulting in the stunted indefinitely vegetative growth characteristic of spring-sown plants (**4**). Morphological characteristics of spring varieties, such as Hard Federation, which are less susceptible to modification by small temperature differences, are greatly affected by differences in day length. By increasing the natural length of the day through artificial illumination, the plants may be so rapidly forced into heading that at maturity they are extremely stunted, with reduced tillering, short spindling culms, and small leaves and heads. By decreasing the length of the light period, large vegetative plants are produced, with increased tillering but reduced yields of grain (**29**). Such variations in type of growth would seem to reflect correlative differences in the chemical organization of the plant.

Early investigators (**43, 30, 31**) attributed the transition from vegetative to reproductive growth to the accumulation of some unnamed organic material acting as a stimulus to flowering. The suggestions followed that the transition is brought about by the accumulation of carbohydrates (**32, 35**) or by an excess of carbohydrates over nitrogenous compounds (**14, 15, 34**).

The relative amounts of carbon and nitrogen compounds in the tissues have been reported to determine many aspects of plant behavior. Not only the general condition of fruitfulness, and of contrasting vegetative development, but also the rate and amount of growth, root development, relation of size of tops to that of roots, resting periods, fruit-bud differentiation, and the phenomena involved in "photoperiodism" have been reported to be correlated with the balance between carbon and nitrogen compounds in the tissues (**3, 23, 33, 46**). The analytical work on wheat includes that of

REID (**41, 42**), who showed that the type of seedling growth is determined by the relative amounts of available carbon and nitrogen compounds; and of HICKS (**21, 22**), who concluded that successive stages of development are initiated by critical values of the carbon/nitrogen ratio, flowering being brought about by a high C/N value.

The purpose of the present investigation was to extend these observations to a chemical characterization of the widely differing growth responses shown by the wheat plant when grown under various conditions of day length and temperature. By growing the winter variety, Turkey, and a spring variety, Hard Federation, under a number of controlled greenhouse environments, many different types of growth were obtained, ranging from the complete sterility of entirely vegetative plants, and the partial sterility resulting from extreme forcing conditions, to normal vigor and grain development. The data obtained are discussed from the standpoint of the direct effects of day length, temperature, light intensity and soil reaction, and also with reference to correlations of chemical composition with type of growth and fruitfulness.

Methods

1. ENVIRONMENTAL CONDITIONS

Seeds of the varieties Hard Federation (C. I. 4733) and Turkey (C. I. 1558), representing respectively extreme types of spring and winter wheats, were sown on December 13, 1929, in each of two similarly situated greenhouses with automatically controlled temperatures. One house was kept at 10°–12° C., a temperature favorable for wheat, the other at 21°–23° C., which is unfavorably high. This high temperature was especially unfavorable for the development of Turkey, whose heading was largely inhibited above 20° C. Temperature control was satisfactorily maintained until the second week of April, when outdoor temperatures became too high. After this date an average difference of 5° C. in the temperature of the two houses was maintained by means of a sprinkler system on the roof of the low-temperature house.

The soil used was a clay-loam compost, 10 inches deep, thoroughly mixed to insure uniformity. Soil moisture was kept as uniform as possible in all the benches.

The plants of the first bench in each house were given a shortened day by means of a heavy black sateen cover, which was in place from 4 P. M. until 8 A. M. the next morning, giving the plants a light period of 8 hours in the middle of the day. The cover was adjusted over a framework to which it was secured at the corners by snap fasteners. It was removed by rolling it off the framework on a rod which constituted one edge of the frame. Neither soil nor air temperatures in the bench were affected appreciably by the cover.

The plants of the second bench in each house were given a light period of 17 to 19 hours by supplementing the natural day with artificial illumination from 4 P. M. until midnight. Three-hundred-watt Mazda-C bulbs in standard deep-bowl reflectors were suspended at 32-inch intervals, 4 feet above the level of the soil. The light intensity at the level of the soil was approximately 100 foot-candles. Thermographs directly beneath the lights recorded an increase in air temperature of 1° C. when the lights were on, but there was no appreciable effect on the soil temperature. As the plants increased in height, the lights were raised to 5 and eventually to 6 feet above the bench.

A third bench, adjacent to the one directly under the lights, received the same period of illumination but the intensity of the supplementary light was only 50 foot-candles at the edge of the bench nearest the lights, and 15 foot-candles in the corners farthest away.

The natural-day control plants of the fourth bench in each house were protected from the lights over the long-day bench by a heavy black sateen curtain hung in the intervening aisle during the period when the lights were on. The natural length of day increased from 9.5 hours at the beginning of the experiment to 15 hours when the last plants were harvested.

In order to determine whether liming would affect the growth and chemical composition of the plants, each bench was divided into two parts by a tight board partition, and commercial hydrated lime was mixed into the soil of the north section at the rate of 4 tons per acre. The reaction of 2:1 water extracts of the limed soil was pH 8.1 as compared with pH 7.0 for the untreated soil.

2. Sampling procedure

Each lot of plants was sampled in the early tillering stage and again at heading time. At the time of the first samplings, the plants were from 4 to 7 weeks old and had no stem tissue, so the material taken for analysis consisted of the entire plants, excepting the roots. The plants were always cut in the morning to avoid the effects of diurnal variations in composition. For the carbohydrate analyses the leaves were immediately ground to a pulp in a Nixtamal mill. Fifty-gram samples were quickly weighed into tared wide-mouthed 500-cc. Erlenmeyer flasks, each containing 0.2 gm. calcium carbonate. The pulp was then covered with enough boiling 95 per cent. alcohol to make the final concentration approximately 80 per cent., and boiled in a water bath for 15 minutes. Similar material was dried for 24 hours in a well ventilated steam-heated oven (55°–60° C.) for nitrogen determinations. A third lot of the leaves was ground through the mill and the juice squeezed through cheese-cloth by hand for pH determinations.

At the time of the second samplings, when the plants were in the heading stage, the leaves were stripped from the culms and those which were dried

or yellow were discarded. In all the samplings of both young and old plants, only green leaves were used. The culms, together with considerable leaf-sheath tissue, were analyzed separately.

At the time of the first samplings the plants of each group, *i.e.*, those subjected to the same temperature and soil reaction but to four different light conditions, were all cut on the same day in order to obtain close comparisons of day-length effects uncomplicated by the uncontrollable factors that differ from day to day. For the second samplings the plants of each lot were cut when the first flowers emerged, on the dates indicated in the tables.

3. Chemical analyses

Carbohydrates.—Reducing sugar, sucrose, and total sugar in the samples preserved in alcohol were extracted according to the following procedure. The original alcohol was decanted through a weighed filter paper into a 1000-cc. volumetric flask. The tissue was then extracted three times with 100-cc. portions of boiling 85 per cent. alcohol. On cooling, a gelatinous precipitate appeared, so the extractions were filtered into the original extract after standing overnight. The volumetric flask was then made up to volume with 85 per cent. alcohol and aliquots were removed into beakers. These aliquots were freed of alcohol by evaporating almost to dryness in a water bath at 60° with a strong current of air. They were then taken up in hot distilled water and cooled after a few minutes by the addition of more cold water. The solutions were cleared with neutral lead acetate and the excess lead precipitated with potassium oxalate.

The reducing values of the cleared solutions were determined by the Shaffer-Hartmann method (**44**), modified slightly with respect to the volumes of solutions used. From previous work on similar material it had been found that sucrose was the only dissacharide present in appreciable amounts. So both sucrose and total sugar were obtained by the hydrochloric acid inversion method of Herzfeld, as given by Browne (**6**, p. 266), and calculated from the reducing values by means of the Munson-Walker sugar tables.

After extraction of the sugars, the residue of each sample was transferred with the filter paper to an aluminum weighing can and weighed after drying for 48 hours at 100° C. The weight of the alcohol-soluble material was obtained by drying aliquots of the extracts on quartz sand in tared containers at 60° C. in a vacuum oven for 8 hours, after previous reduction to air dryness in a current of warm air. The weight of this alcohol-soluble material plus the weight of the extracted and dried residue constituted the total dry weight on which calculations of the sugars were based.

The acid-hydrolyzable material was determined after grinding the dried, extracted residue in an ordinary drug mill, then in a ball mill, until it

passed through an 80-mesh sieve. Two-gram samples, previously freed of water by drying at 100° C., were extracted with 2.5 per cent. H_2SO_4 for 2 hours on a sand bath. After hydrolysis the samples were filtered, neutralized, cleared with neutral lead acetate, and the excess lead removed with potassium oxalate. From the reducing power of aliquots of the cleared solutions, made to volume, the percentages of acid-hydrolyzable material were calculated as glucose.

Sufficient material for starch determinations was available at the heading stage only. One-gram samples of the original residue after extraction of the sugars were digested with a preparation of diastase known as Pangestin, a product of the Digestive Ferments Co. of Detroit, Michigan. After heating to boiling, and filtering, the solutions were made up to a concentration of 2.5 per cent. H_2SO_4 and hydrolyzed for 1 hour by boiling gently on a sand bath. After neutralizing and making to volume, reducing power was determined and the results calculated as percentages of starch.

All analytical procedures were carried out in duplicate. In the case of the glucose determinations, the maximum deviations from the mean of the two duplicates never exceeded an absolute difference of 0.1 per cent., so that differences greater than this in the glucose percentages given in the tables are probably real differences between the samples and not errors due to the method. Owing to the larger percentages of sucrose and total sugars present, their absolute percentage deviations from the mean of the duplicates increased to as much as 0.2, only 5 per cent. of the duplicates showing a greater discrepancy than this. It is therefore fairly safe to assume that, in this material, an absolute difference greater than 0.2 per cent. indicates a real difference in the samples. In the case of the acid-hydrolyzable data, the corresponding limit of accuracy is increased to 0.3, differences of this magnitude or less being obtained in 95 per cent. of the duplicate determinations.

NITROGEN.—Nitrogen, not including nitrates, was determined by the Kjeldahl method on material dried at 55°–60° C., then ground in a Wiley mill. The moisture content of the air-dry material was determined by drying duplicate portions to constant weight (48 hours) in an electric oven. The nitrogen determinations were thus corrected for the moisture content of the samples and expressed as percentages of the oven-dry weights.

The nitrogen values given in the tables are the averages of at least three replications. The maximum deviation from the mean of the three percentages was less than 0.1 in 98 per cent. of the samples, so differences greater than 0.1 per cent. between determinations on different samples are assumed to be significant.

ACIDITY.—Electrometric determinations of the pH values of the juice of the fresh tissues, cut within an hour after the material for the corre-

sponding carbohydrate and nitrogen samples, were made with a Wolf potentiometer and accessory equipment. The system was accurate to within ± 0.01 pH, according to frequent checks with M/20 potassium acid phthalate.

4. Calculations

The difficulty of expressing the analytical data without the distortion inevitably resulting when the dry-weight basis changes during the experiment seems insurmountable. Unless the data are expressed in terms of the absolute weight of material per plant or organ (which in the case of such variable plants as wheat (**9**) requires a large amount of material) they are merely statements of ratios of the constituent under consideration to the total solids, and there is a tendency to interpret changes in the ratio as changes in the numerator of the fraction only. Chibnall (**8**) pointed out that estimation of the diurnal changes in nitrogen from percentages based on the dry weight of the leaf gives inaccurate and misleading results, and recommended that such percentages be calculated on the less variable fresh weight of the tissue. Mason and Maskell (**37**) concluded that the apparent constancy of the fresh-weight basis is largely illusory and that in the absence of specific evidence of constancy, or especially when differential treatment might be expected to affect the moisture relations of the plant, this basis, like the customary dry-weight basis, is unsatisfactory. They suggest that when it can be safely assumed that the variation in the basic dry weight during the experiment is due to variation in labile carbohydrates, a logical procedure is to base analyses on the so-called residual dry weight, *i.e.*, the total dry weight minus these carbohydrates. Changes in nitrogen or ash constituents are assumed to be proportionately too small to produce appreciable effects on the data. The applicability of the method is limited to experiments involving mature tissues and extending over short periods of time during which the residual dry weight can be assumed to be practically constant (**36**).

In the present investigation, use of the fresh-weight basis was unsatisfactory because of uncertainty as to the constancy of the water relations under the different experimental conditions, and also because of the small magnitudes of some of the percentages. The use of the residual dry weight, the most logical in some respects, was precluded by the fact that the experiments were of long duration and involved rapidly growing plants, with every likelihood of variability of constituents other than labile carbohydrates under the different experimental conditions (**36, 37**). The total dry weight was also unsatisfactory because of its obvious inconstancy. Nevertheless, in the absence of a better method, the data are reported on this basis, which has the advantage of being the one most commonly used. It introduces magnifying effects in some instances and compensating effects in

others, but the trends shown by the data as calculated by all three methods are, in general, the same. In table II and III, where a discrepancy in the trend of nitrogen with temperature appeared which seemed related to a concomitant variation in the dry weights, the results as calculated by both residual and total dry weights are reported.

The symbol C-h/N is used in the tables to denote the ratio between the total carbohydrates and the nitrogen percentages.

Growth responses

The control plants with the natural length of day produced the best growth and yield in both the spring and the winter varieties. The plants all grew more vigorously at the low (10°–12°) than at the high (21°–23°) temperature. Thus the best plants of both varieties were the natural-day control plants of the low-temperature house. The degree of injury produced by the unfavorable factors—the lengthened light period, the shortened light period, and the high temperature—differed markedly in the two varieties.

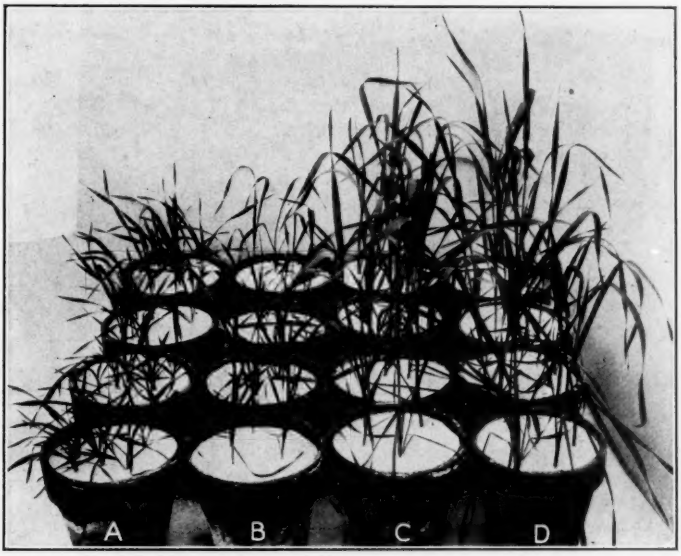

FIG. 1. Hard Federation wheat sown Feb. 25, 1930, photographed at age of 1 month (21°–23° C.):

A, short (8-hour) day
B, natural (12-hour) day
C, long (18-hour) day; supplementary light, 15–50 foot-candles
D, long (18-hour) day; supplementary light, 100 foot-candles.

The long daily light period forced the plants of the spring variety, Hard Federation, into heading so rapidly that at both temperatures there was insufficient vegetative development for normal head and grain formation. The plants were short and stunted in appearance at maturity, with reduced tillering and with small leaves and heads. Plants of Turkey, the winter variety growing beside them, were less sensitive to the forcing action of the long day, and, at the low temperature, appeared normal except for a reduction in number of tillers. In fact, their average weights of grain per head and their average kernel weights exceeded those of the control plants (table I), but their smaller number of tillers lowered their total yield.

TABLE I

EFFECTS OF ENVIRONMENTAL FACTORS ON HEAD AND KERNEL DEVELOPMENT OF HARD FEDERATION AND TURKEY WHEAT, 1930*

DAY LENGTH	SOIL REACTION	HARD FEDERATION		TURKEY	
		10°–12° C.	21°–23° C.	10°–12° C.	21°–23° C.
		Weight of grain from 100 heads			
	pH	*gm.*	*gm.*	*gm.*	
Short (8 hours)	7.0	78.51	40.46	0	n†
	8.1	74.65	34.42	0	n
Natural (9.5–15 hours)	7.0	117.40	88.30	104.40	f
	8.1	135.59	101.89	96.09	f
Long (18 hours)	7.0	3.07	20.68	118.80	f
(15–50 foot-candles)	8.1	3.48	16.94	121.73	f
Long (18 hours)	7.0	7.85	22.85	120.34	f
(100 foot-candles)	8.1	6.73	.....	120.69	f
		Weight of 100 kernels			
Short (8 hours)	7.0	3.40	2.96	.....	.....
	8.1	3.63	2.95	.....	.....
Natural (9.5–15 hours)	7.0	4.92	4.08	3.78	.....
	8.1	4.91	4.02	3.31	.....
Long (18 hours)	7.0	4.51	3.24	4.33	.....
(15–50 foot-candles)	8.1	4.39	3.14	4.12	.....
Long (18 hours)	7.0	4.77	3.33	4.27	.....
(100 foot-candles)	8.1	4.85	.....	4.26	.....

* Temperatures were maintained during first four months only.
†n, no heads formed; f, few heads formed, mostly sterile.

At the low temperature, the short daily light period produced large vegetative plants in both varieties, with increased tillering and leaf development and retarded heading and maturation, as compared with the controls with the natural day. The winter variety was more injured than was the spring variety at this day length, its heads being completely sterile.

At the high temperature, heading in Turkey was almost completely inhibited at every day length, whereas Hard Federation produced almost as much grain at this temperature as at the lower one, although the plants were all shorter.

The extreme susceptibility of Hard Federation to the forcing action of the long day is shown in figures 1 and 2. Owing to the difficulty of

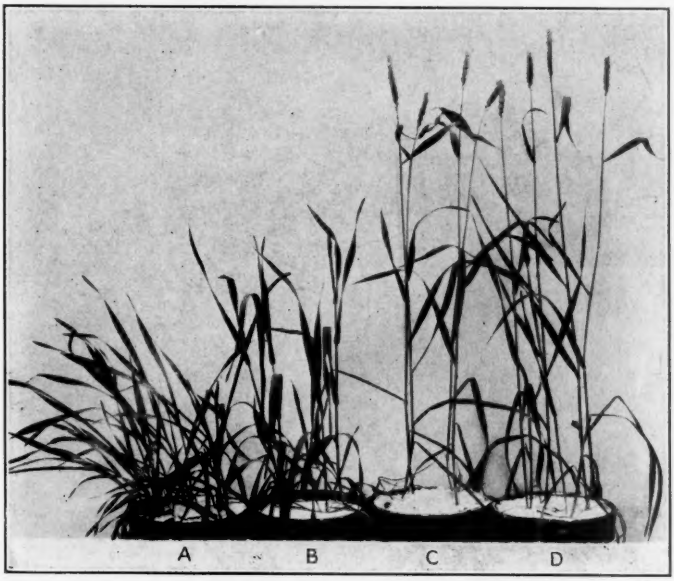

FIG. 2. Hard Federation wheat sown Feb. 25, 1930, photographed at age of 2 months (21°–23° C.):

A, short (8-hour) day
B, natural (13-hour) day
C, long (18-hour) day; supplementary light, 15–50 foot-candles
D, long (18-hour) day; supplementary light, 100 foot-candles.

photographing the plants in the benches, those shown in these figures were grown in pots for the pictures, the soil being covered with a layer of white sand. In figure 1 is shown the rapid development of the long-day plants and the increased tillering of the short-day plants. In figure 2 some of these plants are shown a month later when those of the long day were past flowering. The control plants (*B*) with the natural day were in an early shooting stage, and the short-day plants (*A*) were still in the tillering stage. Subsequently the height of both the short- and the natural-day plants greatly surpassed that of the long-day plants (*C*, *D*).

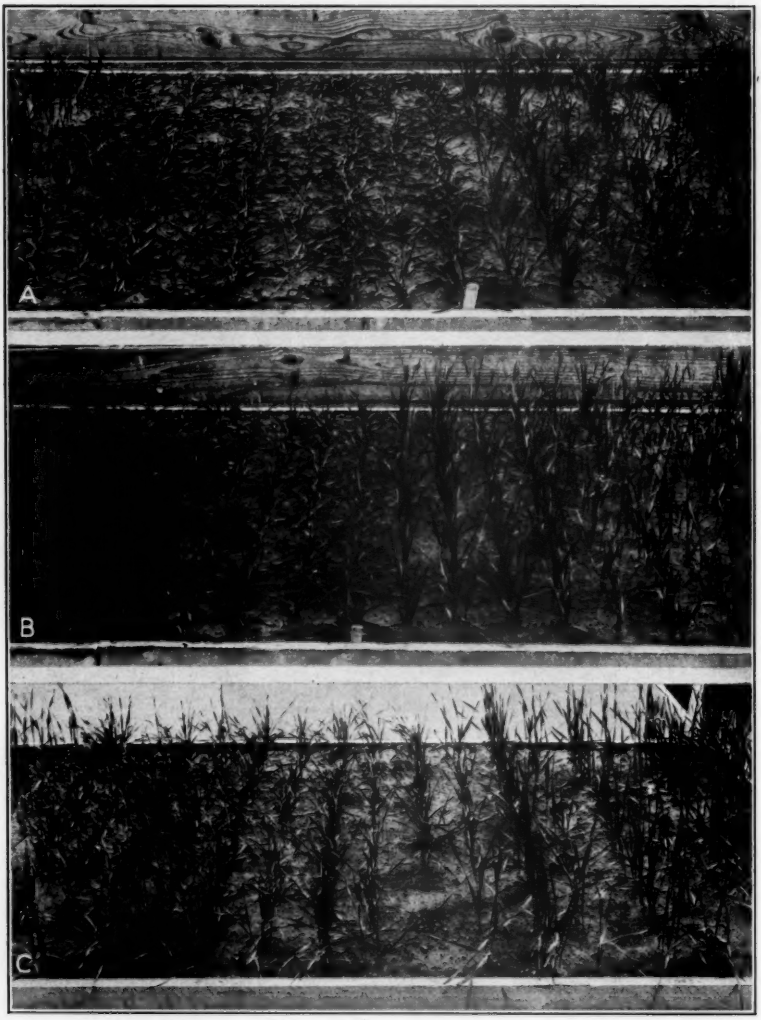

FIG. 3. Turkey (left) and Hard Federation (right), sown Dec. 5, 1929, photographed at age of 1 month (10°–12° C.):
A, short (8-hour) day
B, natural (9.5-hour) day
C, long (16.5-hour) day.

A conspicuous modification of growth habit induced by the long day was the inhibition of the recumbent type of growth, normally characteristic of fall sowings of Turkey and similar varieties during the winter months (**28**). Only at the shorter day lengths, *i.e.*, the artificial one of 8 hours and the natural winter day of about 10 hours, did the plants become recumbent. The resulting difference in the appearance of the plants near the time of the first samplings is shown in figure 3. At the short and at the natural days the Turkey plants are shown in the prostrate condition with the erect plants of Hard Federation beside them (*A, B*). At the long day (*C*) Turkey was as erect as Hard Federation, never having passed through a prostrate stage.

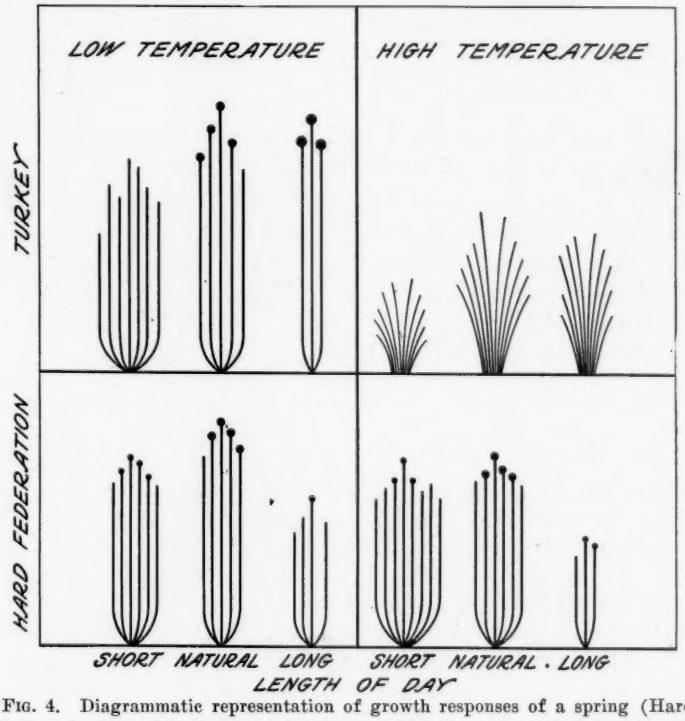

FIG. 4. Diagrammatic representation of growth responses of a spring (Hard Federation) and winter (Turkey) wheat to short (8-hour), natural (9.5 to 15-hour), and long (17-hour) days at low (12° ± 1°) and high (21° ± 1°) temperatures. Heights are drawn to scale from averages. The number of verticals with dots shows the average number of fertile tillers per plant, and those without dots, the sterile tillers. Relative yield per head is roughly shown by small, medium, and large dots on the verticals. Leafy sterile plants of Turkey with few or no culms are represented by groups of spreading lines.

The increase in soil alkalinity from pH 7.0 to pH 8.1 in the limed end of each bench had no appreciable effect on the growth or rate of development of the plants of either variety. The difference in the intensity of the supplementary illumination prevailing in the two long-day benches of each house also produced no visible differences in the plants.

A general characterization of the type of growth of each lot of plants is given in the tables beside the corresponding chemical data. Data on head and kernel development are recorded in table I. Average growth measurements for similar plants grown under approximately the same conditions are represented diagrammatically in figure 4, which shows the relative height, number of fertile and sterile tillers, and relative yield of grain of both varieties under the six different conditions of day length and temperature. Detailed descriptions of these plants are reported elsewhere (**29**).

Results of chemical analyses

1. Effects of day length on carbohydrates, nitrogen, and pH values of wheat leaves

Young plants.—The data in tables II and III show that in young plants of both varieties there was generally a positive correlation between the amount of carbohydrates in the tissues and the length of the daily period of illumination. The glucose fraction varied directly with day length without exception in Turkey (table II), but with less consistency in some groups of Hard Federation (table III). The data on sucrose were not consistently correlated with day length in either variety. The figures for total sugar, acid-hydrolyzable material, and total carbohydrates leave no doubt but that the long day tended to bring about an increase in these compounds and that the short day tended to decrease them. The accumulation of sugar brought about by the 17-hour day was especially great in the low-temperature plants of Turkey (groups 1 and 3 of table II).

The effect of day length on the percentage of nitrogen in the tissues was the opposite of the effect on carbohydrates. Without exception the short light period increased it and the long one decreased it, in comparison with the control plants with the natural day. That the low nitrogen content of the long-day plants might have been responsible for their type of growth is suggested by the similarity between some of their abnormalities—such as reduced tillering, decreased vegetative growth, hastened maturation, and, in Turkey, increased weight of individual kernels—and the abnormalities caused by deficient nitrogen supply (**11**).

The data obtained by Garner, Bacon, and Allard (**18**), showing that shortening the day increases the sugar content of cosmos (which flowers with a short day) while lengthening the day increases the sugar content

of radish (which flowers with a long day), suggest that the effects of day length on carbohydrates vary with the photoperiodic characteristics of the species, or variety, thus offering a possible explanation for some of the conflicting reports in the literature on this subject. Wheat is a long-day plant. The evidence from the present study and from that of ARTHUR, GUTHRIE, and NEWELL (**1**), pointing to a direct relationship between day length and carbohydrate content in wheat, is therefore consistent with this generalization.

The pH values given in tables II and III show that in Hard Federation at the low temperature (groups 5, 7) there were no consistent effects of day length on acidity; but at the high temperature, and, in Turkey, at both temperatures, the shortened light period generally increased and the lengthened one decreased the pH value of the juice. This relation is consistent with GARNER, BACON, and ALLARD'S report (**18**) that, in long-day plants, exposure to a short light period results in the acidity remaining at a low level, while exposure to a long one commonly causes it to increase.

HEADING PLANTS.—In tables IV and V are given the results of analyses for each lot of plants when they reached the heading stage, or, in the case of those which did not head, an advanced age. The plants of the different day lengths constituting each group could not be sampled on the same day, as they had been at the earlier samplings, because of their widely different rates of development. Each lot of plants was therefore sampled when the majority of the heads had emerged from the boot, with the earliest ones flowering.

Environmental conditions had changed considerably for these older plants. The controls, subjected to the natural light period, were receiving from 13 to 15 hours of light per day, depending on the date of cutting, instead of the 10 hours or less that they had received in the winter months. Temperature control had to be discontinued the second week of April, by which time only the most rapidly developing plants of Hard Federation had reached the heading stage. The rest were therefore subjected to considerably higher temperatures for various periods, depending on their dates of heading. The approximately 10° difference between the low- and high-temperature houses, maintained during the first four months, was reduced to 5°. The light intensity had of course increased during the spring months. The environments were thus quite different from those responsible for the results in tables II and III.

Unlike the data on the younger plants, those obtained at heading time did not show the regular trends of increasing carbohydrates and decreasing nitrogen percentages with increasing day length. In Turkey (table IV) there was still a definitely higher total sugar and total carbohydrate concentration in the leaves of the plants with the long day, but this relation no

TABLE II

EFFECTS OF ENVIRONMENTAL FACTORS ON CHEMICAL COMPOSITION OF YOUNG PLANTS OF TURKEY, A WINTER WHEAT
[CARBOHYDRATE AND NITROGEN DATA EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS WHEN NOT INDICATED OTHERWISE]

DAY LENGTH*	DATE OF SAMPLING (1930)	DRY WEIGHT (PER CENT OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	ACID HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN BASED ON		C-h/N	pH OF JUICE	SUBSEQUENT DEVELOPMENT
		TOTAL	RESIDUAL						TOTAL DRY WT.	RESIDUAL DRY WT.			
	Group 1: plants grown at 10°–12° C., neutral soil												
		%	%	%	%	%	%	%	%	%			
Short (8)	2/1	17.82	14.49	2.32	5.71	8.05	10.66	18.71	6.21	7.64	3.01	6.12	Vegetative; sterile heads
Natural (10)	2/1	17.92	14.25	2.79	6.01	8.85	11.64	20.49	5.42	6.82	3.78	6.04	Normal; good yield
Long (17)†	2/1	17.57	12.78	4.46	9.26	13.85	13.43	27.28	4.57	6.29	5.97	5.96	Almost normal; good yield
Long (17)‡	2/1	17.55	12.86	4.49	8.82	13.43	13.28	26.71	4.46	6.09	5.99	5.96	Almost normal; good yield
	Group 2: plants grown at 21°–23° C., neutral soil												
Short (8)	1/10	12.42	10.84	1.12	1.75	2.83	10.14	12.77	5.56	6.38	2.30	5.99	Leafy growth; sterile
Natural (10)	1/10	12.97	11.02	1.63	2.50	4.09	10.94	15.03	5.29	6.22	2.84	5.88	Leafy growth; almost sterile
Long (17)†	1/10	11.87	9.95	2.28	2.11	4.33	11.85	16.18	4.50	5.37	3.60	5.86	Leafy growth; almost sterile
Long (17)‡	1/10	13.21	10.98	2.16	2.21	4.34	12.61	16.95	4.60	5.53	3.68	5.82	Leafy growth; almost sterile

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE II.—(*Continued*)

Day length*	Date of sampling (1930)	Dry weight (per cent of wet weight)		Glucose	Sucrose	Total sugar	Acid hydrolyzable material including starch	Total carbohydrates	Nitrogen based on		C-h/N	pH of juice	Subsequent development
		Total	Residual						Total dry wt.	Residual dry wt.			
		Group 3: plants grown at 10°–12° C., alkaline soil											
		%	%	%	%	%	%	%	%	%			
Short (8)	1/25	16.97	13.56	2.43	7.36	9.86	10.24	20.10	5.39	6.75	3.73	6.07	Vegetative; sterile heads
Natural (10)	1/25	16.87	13.30	2.64	7.20	9.87	11.30	21.17	5.14	6.52	4.12	5.98	Normal; good yield
Long (17)†	1/25	17.06	12.91	3.59	8.78	12.44	11.91	24.35	4.61	6.10	5.28	6.00	Almost normal; good yield
Long (17)‡	1/25	16.98	12.63	3.99	9.22	13.40	12.23	25.63	4.48	6.02	5.72	5.95	Almost normal; good yield
		Group 4: plants grown at 21°–23° C., alkaline soil											
Short (8)	1/11	11.84	10.26	0.82	0.80	1.58	11.81	13.39	5.67	6.55	2.36	6.10	Leafy growth; sterile
Natural (10)	1/11	11.96	10.44	1.08	0.36	1.39	11.31	12.70	5.22	5.99	2.43	6.05	Leafy growth; almost sterile
Long (17)†	1/11	11.52	9.77	1.58	0.83	2.32	12.91	15.23	4.90	5.78	3.11	5.96	Leafy growth; almost sterile
Long (17)‡	1/11	11.90	9.98	1.48	0.84	2.20	13.98	16.18	4.86	5.80	3.33	5.94	Leafy growth; almost sterile

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE III

EFFECTS OF ENVIRONMENTAL FACTORS ON CHEMICAL COMPOSITION OF YOUNG PLANTS OF HARD FEDERATION, A SPRING WHEAT
[CARBOHYDRATE AND NITROGEN DATA EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS WHEN NOT INDICATED OTHERWISE]

DAY LENGTH*	DATE OF SAMPLING (1930)	DRY WEIGHT (PER CENT. OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN BASED ON		C-h/N	pH OF JUICE	SUBSEQUENT DEVELOPMENT
		TOTAL	RESIDUAL						TOTAL DRY WT.	RESIDUAL DRY WT.			
	Group 5: plants grown at 10°–12° C., neutral soil												
		%	%	%	%	%	%	%	%	%			
Short (8)	1/29	13.67	11.59	1.22	2.66	3.85	11.37	15.22	6.30	7.43	2.42	6.08	Vegetative; reduced yield
Natural (10)	1/29	14.27	12.01	1.50	3.79	5.28	10.58	15.86	5.95	7.07	2.67	6.03	Normal; good yield
Long (17)†	1/29	13.94	11.30	1.89	3.88	5.79	13.16	18.95	5.34	6.59	3.55	6.08	Stunted; poor yield
Long (17)‡	1/29	14.80	11.88	1.71	3.80	5.54	14.20	19.74	5.04	6.28	3.92	6.07	Stunted; poor yield
	Group 6: plants grown at 21°–23° C., neutral soil												
Short (8)	1/9	11.86	10.00	2.11	2.52	4.55	11.63	15.71	5.56	6.64	2.83	5.98	Vegetative; poor yield
Natural (10)	1/9	11.77	9.96	1.42	2.02	3.47	11.85	15.32	5.21	6.15	2.94	5.93	Fair growth; moderate yield
Long (17)†	1/9	11.52	9.52	2.34	1.92	4.40	12.95	17.35	4.72	5.72	3.68	5.87	Stunted; poor yield
Long (17)‡	1/9	11.79	9.60	1.84	2.12	3.95	14.65	18.56	4.58	5.63	4.05	5.87	Stunted; poor yield

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE III.—(*Continued*)

Day length*	Date of sampling (1930)	Dry weight (per cent. of wet weight)		Glucose	Sucrose	Total sugar	Acid-hydrolyzable material including starch	Total carbohydrates	Nitrogen based on		C-h/N	pH of juice	Subsequent development
		Total	Residual						Total dry wt.	Residual dry wt.			
Group 7: plants grown at 10°–12° C., alkaline soil													
		%	%	%	%	%	%	%	%	%			
Short (8)	1/28	13.98	12.04	0.87	0.72	1.62	12.26	13.88	6.51	7.56	2.13	6.09	Vegetative; reduced yield
Natural (10)		13.63	11.63	1.29	1.02	2.25	12.50	14.75	6.31	7.40	2.34	6.10	Normal; good yield
Long (17)†		13.82	11.47	1.20	1.28	2.44	14.57	17.01	5.38	6.47	3.16	6.08	Stunted; poor yield
Long (17)‡		13.26	10.84	1.33	1.11	2.36	15.92	18.28	5.25	6.43	3.48	6.11	Stunted; poor yield
Group 8: plants grown at 21°–23° C., alkaline soil													
Short (8)	1/13	11.29	9.82	1.05	0.35	1.35	11.73	13.08	5.84	6.72	2.24	5.94	Vegetative; poor yield
Natural (10)		11.71	10.02	1.55	0.79	2.29	12.11	14.40	5.66	6.61	2.54	5.95	Fair growth; moderate yield
Long (17)†		11.67	9.80	1.68	0.80	2.44	13.60	16.04	4.98	5.93	3.22	5.90	Stunted; poor yield
Long (17)‡		11.22	9.37	1.75	1.10	2.82	13.70	16.52	4.78	5.73	3.46	5.87	Stunted; poor yield

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE IV

CHEMICAL COMPOSITION OF LEAVES AND CULMS OF TURKEY WHEAT IN THE HEADING STAGE, GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

[CARBOHYDRATES AND NITROGEN EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS]

DAY LENGTH*	DATE OF SAMPLING (1930)	TISSUE	DRY WEIGHT (PER CENT. OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	STARCH	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN	C-h/N	pH OF JUICE	CONDITION OF PLANTS
			TOTAL	RESIDUAL										
	Group 1: low temperature, neutral soil													
			%	%	%	%	%	%	%	%	%			
Short (8) ...	6/26	Leaves	28.50	22.38	1.75	4.40	6.20	1.42	15.25	21.45	3.66	5.86	5.62	Poor; sterile heads
		Culms	27.12	17.67	9.03	7.28	16.33	2.26	18.53	34.86	1.46	23.9	5.87	
Natural (14)	5/15	Leaves	23.58	18.70	1.02	3.14	4.22	1.58	16.57	20.76	3.23	6.43	5.78	Normal; high yield
		Culms	22.36	15.87	3.40	3.31	6.68	2.08	22.38	29.06	0.74	39.3	5.83	
Long (19)† ...	4/25	Leaves	23.78	17.95	1.61	7.49	9.21	2.55	15.32	24.50	3.84	6.38	5.90	Good; high yield
		Culms	27.27	17.20	6.12	12.71	18.90	2.81	18.05	36.95	1.18	31.3	5.83	
Long (19)‡ ...	4/26	Leaves	24.78	18.63	1.86	7.45	9.44	2.43	15.36	24.71	3.63	6.81	5.97	Good; high yield
		Culms	27.62	17.50	5.43	13.12	18.64	2.80	18.03	36.67	1.00	36.7	5.94	
	Group 2: high temperature, neutral soil													
Short (8)	6/19	Leaves	18.60	15.52	1.33	2.73	4.04	1.56	12.50	16.54	4.09	4.04	5.66	Poorly vegetative; no heads
Natural (15)	6/19	Leaves	20.71	16.54	2.80	2.30	5.12	2.13	15.00	20.12	3.05	6.60	5.52	Poorly vegetative; few heads
Long (19)† ...	6/19	Leaves	24.00	19.16	3.32	2.11	5.44	2.00	14.71	20.15	3.24	6.22	5.41	Poorly vegetative; few heads
Long (19)‡ ...	6/19	Leaves	23.88	18.56	3.95	2.83	6.70	2.20	15.61	22.31	3.47	6.43	5.41	Poorly vegetative; few heads

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE IV—(*Continued*)

Day length*	Date of sampling (1930)	Tissue	Dry weight (per cent. of wet weight)		Glucose	Sucrose	Total sugar	Starch	Acid-hydrolyzable material including starch	Total carbohydrates	Nitrogen	C-h/N	pH of juice	Condition of plants
			Total	Residual										
Group 3: low temperature, alkaline soil														
			%	%	%	%	%	%	%	%	%			
Short (8)	6/26	Leaves	29.70	23.37	1.44	3.56	5.03	0.88	16.30	21.33	3.45	6.18	5.60	Poor; sterile heads
		Culms	27.18	17.49	8.35	8.37	16.75	2.19	18.93	35.68	1.57	22.7	5.82	
Natural (14)	5/15	Leaves	23.28	18.40	1.73	3.72	5.48	1.62	15.47	20.95	3.44	6.09	5.65	Normal; high yield
		Culms	26.84	17.89	5.84	5.84	11.70	2.27	21.64	33.40	0.85	39.3	5.67	
Long (19)†	4/24	Leaves	26.46	19.26	2.07	9.25	11.39	2.69	15.82	27.11	3.65	7.43	5.82	Good; high yield
		Culms	29.34	17.95	6.32	13.31	19.81	3.17	19.00	38.81	0.99	39.2	5.87	
Long (19)‡	4/23	Leaves	24.88	18.38	2.12	7.90	10.07	2.77	16.06	26.13	3.58	7.30	5.88	Good; high yield
		Culms	27.84	17.91	7.57	8.71	16.31	2.77	19.34	35.65	0.95	37.5	5.81	
Group 4: high temperature, alkaline soil														
Short (8)	6/19	Leaves	18.45	15.25	1.70	2.64	4.34	2.32	13.04	17.38	4.25	4.09	5.59	Poorly vegetative; no heads
Natural (15)	6/19	Leaves	24.08	19.31	2.37	2.21	4.57	2.04	15.25	19.82	3.09	6.41	5.32	Poorly vegetative; few heads
Long (19)†	6/19	Leaves	23.35	18.84	3.17	2.31	5.46	1.71	13.84	19.29	3.41	5.66	5.40	Poorly vegetative; few heads
Long (19)‡	6/19	Leaves	23.05	18.43	3.21	2.36	5.58	1.51	14.45	20.03	3.25	6.16	5.31	Poorly vegetative; few heads

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.

TABLE V

CHEMICAL COMPOSITION OF LEAVES AND CULMS OF HARD FEDERATION WHEAT IN THE HEADING STAGE, GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS
[CARBOHYDRATES AND NITROGEN EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS]

DAY LENGTH*	DATE OF SAMPLING (1930)	TISSUE	DRY WEIGHT (PER CENT. OF WET WEIGHT)		GLUCOSE	SUCROSE	TOTAL SUGAR	STARCH	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN	C-h/N	pH OF JUICE	CONDITION OF PLANTS
			TOTAL	RESIDUAL										
	Group 5: low temperature, neutral soil													
			%	%	%	%	%	%	%	%	%			
Short (8) ...	5/13	Leaves	21.44	16.89	1.01	2.42	3.42	1.69	17.86	21.28	3.48	6.11	5.95	Fair growth; reduced yield
		Culms	18.67	14.41	1.54	1.25	2.82	1.92	20.00	22.83	1.01	22.6	5.79	
Natural (13)	4/10	Leaves	22.36	17.07	1.91	5.65	7.63	2.18	16.09	23.67	3.61	6.56	5.99	Normal; good yield
		Culms	22.35	15.15	6.01	6.57	12.62	2.89	19.74	32.24	.89	36.2	5.79	
Long (18)† ...	3/3	Leaves	22.70	18.49	0.97	5.99	7.05	1.92	11.46	18.53	4.13	4.49	6.32	Stunted; very low yield
		Culms	22.41	15.67	3.22	7.52	10.77	2.68	19.32	30.09	1.45	20.8	6.25	
Long (18)‡ ...	3/1	Leaves	21.68	17.30	1.32	5.98	7.38	2.81	12.87	20.25	4.10	4.94	6.37	Stunted; very low yield
		Culms	23.66	16.38	3.83	8.09	11.95	2.40	18.83	30.78	1.55	19.9	6.20	
	Group 6: high temperature, neutral soil													
Short (8)	4/19	Leaves	21.40	17.17	0.73	1.99	2.71	1.50	17.07	19.78	3.52	5.62	6.01	Poor growth; low yield
		Culms	18.79	14.42	1.74	1.38	3.16	2.19	20.11	23.27	1.61	14.5	5.96	
Natural (13)	3/28	Leaves	22.82	17.54	1.91	6.21	8.20	1.59	14.97	23.17	3.54	6.55	6.03	Fair growth; moderate yield
		Culms	24.55	16.47	6.94	6.82	13.79	2.68	19.14	32.93	1.36	24.2	5.99	
Long (17)§ ...	1/31	Leaves	17.32	14.20	0.98	4.87	5.86	1.79	12.08	17.94	3.98	4.51	6.08	Stunted; poor yield
		Culms	17.81	13.57	2.25	1.32	3.48	2.10	20.32	23.80	2.10	11.3	.....	

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.
§ Long-day plants of both light intensities taken together to make up one sample.

TABLE V—(*Continued*)

Day length*	Date of sampling (1930)	Tissue	Dry weight (per cent. of wet weight)		Glucose	Sucrose	Total sugar	Starch	Acid-hydrolyzable material including starch	Total carbohydrates	Nitrogen	C-h/N	pH of juice	Condition of plants
			Total	Residual										
Group 7: low temperature, alkaline soil														
			%	%	%	%	%	%	%	%	%			
Short (8)	5/13	Leaves	21.00	16.97	0.87	2.44	3.30	2.00	15.88	19.18	3.61	5.31	5.93	Fair growth; reduced yield
		Culms	17.08	12.95	2.07	1.19	3.28	1.57	20.93	24.21	1.29	18.8	5.80	
Natural (13)	4/15	Leaves	21.48	17.29	0.92	3.62	4.54	1.81	14.97	19.51	4.07	4.79	5.99	Normal; good yield
		Culms	20.25	14.91	4.90	1.84	6.65	2.24	19.72	26.37	1.27	20.7	6.00	
Long (18)† ...	3/3	Leaves	21.44	17.22	0.89	5.69	6.65	2.38	13.00	19.65	4.51	4.36	6.15	Stunted; very low yield
		Culms	22.72	16.30	3.19	6.63	9.80	2.70	18.48	28.28	1.73	16.3	6.23	
Long (18)‡ ...	3/1	Leaves	21.54	17.26	1.19	5.94	7.18	2.17	12.66	19.73	4.40	4.48	6.30	Stunted; very low yield
		Culms	23.40	16.52	3.93	6.88	10.77	2.58	18.65	29.42	1.72	17.1	6.20	
Group 8: high temperature, alkaline soil														
Short (8)	4/21	Leaves	21.06	16.48	0.82	3.66	4.50	1.25	17.30	21.80	3.46	6.30	6.12	Poor growth; low yield
		Culms	19.01	14.40	1.93	2.10	3.93	2.25	20.40	24.33	1.63	14.9	6.00	
Natural (13)	4/2	Leaves	21.92	17.47	1.69	4.02	5.72	1.62	14.58	20.30	3.73	5.44	5.99	Fair growth; moderate yield
		Culms	21.35	15.36	4.94	4.50	9.37	2.15	18.72	28.09	1.46	19.2	6.10	
Long (17)§ ...	1/31	Leaves	17.48	14.44	0.80	4.85	5.68	.....	11.69	17.37	4.54	3.83	6.08	Stunted; poor yield
		Culms	16.37	12.28	2.65	0.94	3.42	2.58	21.57	24.95	2.21	11.3	.....	

* Numbers in parentheses indicate hours.
† 15–50 foot-candles.
‡ 100 foot-candles.
§ Long-day plants of both light intensities taken together to make up one sample.

longer appeared consistently in the data for Hard Federation (table V). In the latter variety the leaves of the 8-hour-day plants still had the lowest sucrose and total sugar values in every group, but they had the highest percentages of acid-hydrolyzable carbohydrates. In fact, the acid-hydrolyzable fraction of the leaves of Hard Federation decreased as regularly with increasing day length in every group of the heading plants as it had increased in the case of the younger plants of table III.

The pH values of the leaf juice of the heading plants of tables IV and V were highest in the case of the rapidly maturing plants of the long day. This relation has no significance so far as day length effects are concerned, inasmuch as the long-day plants were much the youngest at the time of cutting. The pH value decreases with age during the maturation period (**24, 25, 26**). The tendency of the carbohydrates to increase and the nitrogen percentages to decrease with age may have similarly obscured the relations between these constituents and day length that had appeared so distinctly at the earlier growth stage.

A marked difference between the chemical composition of the leaves and culms is shown by the carbohydrate and nitrogen percentages given in tables IV and V. The culms of both varieties under all the different environmental conditions were characterized by relatively high carbohydrate and low nitrogen percentages. The glucose percentages were generally from two to three times as high in the culms as in the leaves. The percentages of acid-hydrolyzable material were also consistently higher in the culms. The nitrogen content of the culms was not over one-half and usually but one-third or less of the amount in the leaves. There was but little difference in the pH values.

Outdoor experiments.—Through the courtesy of Mr. H. A. Allard of the Bureau of Plant Industry, space was made available for growing some wheat plants under outdoor conditions identical with those obtaining in his day-length investigations (**16, 17**). Plants of Hard Federation were grown in deep flats placed on trucks which were pushed in and out of light-tight houses at stated hours each day so as to provide 8-, 10-, and 12-hour light periods. The control plants with the natural day, which increased from 12 to 14 hours during the experiment, were left in the open continuously. The plants from each condition were cut on May 9, 1930, in the tillering stage, at the age of 7 weeks. The culms were just beginning to elongate at the two shorter day lengths, and some had reached heights of as much as 6 cm. at the two longer ones. Analyses of the leaves are reported in table VI.

The data show the same increase in carbohydrates and decrease in nitrogen with increasing length of the daily light period as shown by the young greenhouse plants of tables II and III.

TABLE VI

EFFECTS OF DAY LENGTH ON CARBOHYDRATES, NITROGEN, AND pH VALUES OF THE LEAVES OF YOUNG, OUTDOOR HARD FEDERATION PLANTS SOWN MARCH 24, CUT MAY 9, 1930
[CARBOHYDRATE AND NITROGEN DATA EXPRESSED AS PERCENTAGES OF TOTAL DRY WEIGHTS]

DAY LENGTH	DRY WEIGHT (PER CENT. OF WET WEIGHT)	GLUCOSE	SUCROSE	TOTAL SUGAR	ACID-HYDROLYZABLE MATERIAL INCLUDING STARCH	TOTAL CARBOHYDRATES	NITROGEN	C–h/N	pH OF JUICE
hrs.	%	%	%	%	%	%	%		
8	13.42	2.40	2.50	4.82	10.37	15.55	4.90	3.17	5.74
10	13.63	2.52	3.36	5.88	10.36	16.24	4.68	3.47	5.73
12	15.08	2.70	2.62	5.31	12.07	17.39	4.04	4.30	5.72
14 (control)	15.66	2.94	2.98	5.92	13.13	19.05	3.97	4.80	5.69

The carbohydrate percentages in table VI are of the same order of magnitude as those shown by table III to characterize the young greenhouse plants of this variety. The nitrogen percentages and the pH values were lower than in the greenhouse plants. Data of other experiments also have shown a general tendency toward lower nitrogen and pH values, and higher dry weights and sugar percentages, in field plants as compared with greenhouse plants, although the differences are not large.

2. EFFECTS OF INTENSITY OF SUPPLEMENTARY LIGHT

The appearance and rate of development of the plants immediately under the lights supplying the supplementary illumination for the long-day condition, where the light intensity was approximately 100 foot-candles, did not differ from that of the plants in the adjacent bench in which the light intensity was only 15 to 50 foot-candles. Height, tillering, dates of heading, and maturation of both varieties were alike in the two benches. On analysis, however, it was found that there was a small increase in total carbohydrates in the plants with the more intense supplementary illumination in all but one of the eight groups in tables II and III. None of the sugar fractions showed this relation consistently. Thus the increase was largely due to increased percentages of the acid-hydrolyzable material.

The percentage of nitrogen in the long-day plants with the higher supplementary light intensity was generally slightly lower than in those with the lower intensity. There were no differences attributable to this factor in the pH values.

In the older plants of tables IV and V there were no consistent differences in any measurement attributable to the difference in light intensity.

3. Effects of soil reaction

In all but the low-temperature groups of Turkey (groups 1 and 3), the data for both varieties in tables II and III showed markedly lower carbohydrate percentages in the plants of the more alkaline soil (pH 8.1) than in those of corresponding day lengths and temperatures from the neutral soil (pH 7.0). The total sugar percentages for the unlimed plants were approximately twice those of corresponding plants from the limed soil. The difference was mostly due to a reduction in sucrose in the limed plants, although there was an appreciable reduction in glucose also. Bystrikov (**7**) found that increases in soil alkalinity between pH 5.7 and 7.8 brought about progressive decreases in monosaccharides, although his total sugar percentages were not affected.

The acid-hydrolyzable material was not reduced by liming. In fact, there was frequently a slight increase in this fraction in the plants of those groups whose sugar contents were reduced, thus accounting for the proportionately lesser reductions in the total carbohydrate figures than in the sugar percentages.

The young plants of the limed soil with the low sugar percentages generally had somewhat higher nitrogen percentages. These data, including those of the low-temperature plants of Turkey which showed neither the decrease in sugar nor the increase in nitrogen with the limed soil, constitute an illustration of the frequently found inverse relation between the carbohydrate and nitrogen percentages.

The data for the older plants (tables IV and V) showed no consistent effects of soil reaction on these constituents.

There were no consistent differences in the pH values for corresponding plants from the limed and unlimed soils, either in the case of the young plants (tables II and III) or in those that were heading (tables IV and V). In some groups (table III) the values for corresponding plants from the limed and unlimed ends of the benches were alike within a few hundredths of a pH unit. It has been suggested previously (**24**) that liming the soil probably increases the pH value only when it favorably affects the growth and vigor of the plant. In the present experiment the lime had no visible effect on growth or rate of development, so an absence of effect on the pH value might therefore be expected.

4. Effects of temperature

Young plants.—Table II shows a relatively enormous accumulation of sugar at the low temperature in the plants of the winter variety, at all day

lengths. In the neutral soil the low-temperature plants had from two to more than three times as much total sugar, and those in the limed soil six to eight times as much, as did the corresponding plants grown at temperatures about 10° higher. Both glucose and sucrose were higher in the low-temperature plants, but the difference in sucrose was the more extreme. The acid-hydrolyzable fraction did not differ appreciably at the two temperatures. Evidently the hemicellulose, which constitutes the largest part of this fraction, is more easily affected by day length than by temperature.

The low-temperature plants of the spring variety (table III) did not have the high carbohydrate contents so characteristic of the winter variety growing beside them. In fact, there was no significant difference between the low- and high-temperature plants of this variety with respect to any of the carbohydrate fractions. Thus it would seem that the carbohydrate metabolism of Turkey was more affected by both temperature and day length than was that of Hard Federation.

In Hard Federation (table III) the percentage of nitrogen was less at the high than at the low temperature in every group. In Turkey (table II) this relation did not always hold for percentages based on the total dry weight of the tissues, because the large accumulation of carbohydrates, especially in the long-day plants, resulted in disproportionately high total dry weights for the plants of the low-temperature groups of this variety (groups 1 and 3). While this difference in the dry weights merely reduced the contrast in the carbohydrate percentages at the two temperatures, in some groups it completely obscured the usual difference in nitrogen found in preceding experiments—and in the other variety of the present experiment (table III)—in which there were no large accumulations of carbohydrates at the low temperature. When the inequalities in the dry weights were partially corrected by calculating the data on the residual dry-weight basis, the usual inverse correlation of nitrogen with temperature appeared consistently in Turkey as well as in Hard Federation. This relationship was shown also by the calculations based on the fresh weight, not reported in the tables.

The pH values of the juice of plants from corresponding day lengths were lower at the high than at the low temperature, excepting those of Turkey from the alkaline soil (groups 3 and 4 of table II). In previous experiments (**27**), and in others not reported here, lower values were consistently obtained at the higher temperatures, the difference being especially marked in Turkey. The evidence has indicated that increasing the temperature above the optimum for wheat tends to increase the acidity of the leaf juice by an amount which varies with the susceptibility of the particular variety to high-temperature injury (**27**).

HEADING PLANTS.—In the older plants of Hard Federation (table V),

as in the young plants of this variety (table III), there were no definite effects of temperature on the carbohydrate content. In the case of Turkey (table IV), however, the low-temperature plants continued to have the higher carbohydrate percentages, as they had had in the tillering stage (table II). The high-temperature plants of this variety (groups 2 and 4 of table IV), which failed to head and were cut while still in a leafy tillering stage after most of the others had been harvested, were generally characterized by relatively low concentrations of sucrose and relatively high concentrations of glucose.

Neither the nitrogen nor the pH values showed the differences due to temperature which had been so apparent in the young plants. The general absence of positive environmental effects on the chemical constituents of the older plants no doubt was due partly to the procedure of cutting the plants at different times as each lot reached the heading stage. It seems probable (**12, 13, 47, 48, 49**) that, at temperatures below 20° C., where wheat produces the best growth, the amounts of carbohydrates and nitrogenous compounds in the tissues are generally highest, but that exceptions to the generalization will be found as long as there are uncontrolled factors in the experiment.

Discussion

The problem in the foreground throughout these investigations, and the one which initiated them, was the determination of the chemical organization responsible for, or associated with, a particular type of growth. The various conditions of day length and temperature produced widely different types of plants, varying from the small, rapidly maturing plants of the spring variety at the long day to large, slowly maturing, vegetative types at the short day; from vigorous plants with a normal yield of grain at the natural day and low temperature to vegetative plants with culm elongation completely inhibited, such as those of the winter variety at the high temperature.

The data in tables II and III show that the leaves of the young, rapidly developing plants of the long day, which subsequently flowered at an abnormally early age, had the highest carbohydrate and lowest nitrogen percentages, and that those of the highly vegetative plants of the short day had the highest nitrogen and the lowest carbohydrate percentages. The accelerated heading at the long day was associated with very low yield of grain in Hard Federation, although with excellent yield in Turkey; and the vegetative condition at the short day was associated with fair yield in Hard Federation and with sterility in Turkey (table I). Thus there was no relation between the carbohydrate or nitrogen percentages and the subsequent yields of grain.

In the older plants of tables IV and V the carbohydrate and nitrogen percentages of the leaves bore no consistent relation to the type of growth. The leaves of the rapidly maturing long-day plants of Hard Federation (table V), which gave very low yields of grain (table I), had somewhat higher nitrogen values than the other plants of their respective groups; but the differences do not seem large enough to offer a satisfactory explanation of the extreme differences in growth habit and grain yield, and in fact are more probably due to the younger age at which the plants headed and were sampled. Nor were there any distinctive relationships characterizing the leaves of the vegetative short-day plants of Turkey whose heads failed to flower.

The leaves of the high-temperature plants of Turkey (groups 2 and 4 of table IV), most of which failed to produce heads and which were sampled at a late date while still in an abnormal leafy condition from which the plants never emerged, had total carbohydrate and nitrogen percentages of the same order of magnitude as were found in the leaves of the normal heading plants of the other groups. However, all of these sterile plants except those of the short day were characterized by unusually high glucose and low sucrose values. In the heading plants of both varieties the percentages of sucrose in the leaves were considerably higher than those of glucose; but in these vegetative, non-heading plants there was more glucose than sucrose in the leaves of all except the short-day plants. It is not believed that this relation has any general significance in connection with the tendency of the plant to produce grain, because there was no correlation of the glucose/sucrose ratio with fruitfulness in the other groups where poor yields were due to unfavorable light periods instead of to high temperatures.

Thus no consistent correlations were found between the grain production of the plants and the analyses of the leaves. However, the culm analyses of Hard Federation showed that the highest glucose concentration occurred consistently in those plants grown with the natural day where grain yield was highest (table V). The nitrogen percentages were generally somewhat lower in these culms than in those of the less fruitful plants of the short and long days. Unfortunately for a generalization, however, the culms of the heading plants of Turkey (groups 1 and 3 of table IV) did not show a correlation of glucose with fruitfulness, although the nitrogen percentages were again lower in the fertile culms of the natural- and long-day plants than in the sterile ones of the short-day plants.

NIGHTINGALE (**39**) found that the growth responses of tomato, salvia, and several other horticultural plants to different day lengths were correlated with the relative rather than with the absolute amounts of carbohydrates and nitrate-free nitrogen they contained. Other investigators

(**10, 38, 45, 46**) have expressed the opinion that the carbohydrate/nitrogen ratio would be found to explain the day-length responses of the plants with which they were working. From the chemical analyses of wheat, barley, oats, millet, and other plants made by ARTHUR, GUTHRIE, and NEWELL (**1**) and by BORODIN (**5**) however, it would seem that the effects of day length are not brought about by its effect on the carbohydrate/nitrogen ratio. Other evidence of limitations of the theory in explaining particular growth responses in terms of specific carbohydrate/nitrogen ratios has accumulated (**2, 19, 20, 40, 50**), suggesting that under many circumstances the proportionate amounts of these two classes of compounds reveal the physiological balance of the plant in only a general way.

In the present investigation the relatively low carbohydrate-nitrogen ratios of the leaves of the young plants which were subsequently more fruitful than those with higher ratios (table III), the normally high ratios found in the leaves of the vegetative high-temperature plants whose heading was largely inhibited (groups 2 and 4 of table IV), and the generally negative results with the leaves of the older plants, are not in accordance with the correlations which have sometimes been found between this ratio and fruitfulness in other plants. It may be significant that in the leaves of the young plants of both varieties there was always within each group a direct correlation of the magnitude of the ratio with rate of development; *i.e.*, the highest ratios in both varieties characterized the long-day plants, whose flowering was abnormally accelerated and whose vegetative development was inadequate, and the lowest ratios the highly vegetative short-day plants whose development was retarded; but it should be pointed out that since the plants of each group were sampled on the same day the long-day plants were in a more advanced stage of development. Only in the culms was there a consistent relationship between the magnitude of the ratio and the amount of grain produced by the plant (*cf.* table I). In Hard Federation, the culms of the long-day plants which produced the least grain had, without exception, the lowest ratios of each group, and those of the natural-day plants with the highest grain production had the highest ratios. In Turkey, both the natural- and the long-day plants at the low temperature gave good yields of grain, and the carbohydrate/nitrogen ratios of their culms were much higher than were those of the short-day plants which were sterile.

Summary

1. The leaves of young wheat plants, sampled at the same age in the tillering stage, had the highest carbohydrate and lowest nitrogen percentages at the long day, which accelerated culm elongation and flowering in both varieties investigated (Hard Federation, a spring wheat, and Turkey,

the winter variety). The lowest carbohydrate and highest nitrogen percentages were found at the short day, which retarded heading in both varieties and resulted in large vegetative plants, with reduced yield of grain in Hard Federation and complete sterility in Turkey (tables II, III, and VI).

2. Supplementary light of relatively high intensity (100 foot-candles) resulted in higher percentages of acid-hydrolyzable material, and consequently of total carbohydrates, and lower percentages of nitrogen, than the weaker light (15 to 50 foot-candles). There were no effects of this difference in light intensity on the external appearance or rate of development of the plants (tables II and III).

3. The sugar content of the young plants of the limed plots with a soil reaction of pH 8.1 was generally much reduced, and the acid-hydrolyzable material somewhat increased, compared with that of plants of the neutral soil. The nitrogen percentages were generally highest in the plants whose sugar content was reduced. The difference in soil reaction produced no visible effects on the appearance or rate of development of the plants (tables II and III).

4. The young plants of Turkey contained much more sugar, largely sucrose, at the low temperature where growth was normal than at the high temperature where the plants failed to head. In the case of Hard Federation, which headed and yielded fairly well at both temperatures, there was no significant difference in any of the carbohydrate fractions due to temperature. In neither variety was there any difference, attributable to temperature, in the acid-hydrolyzable material (tables II and III).

5. The nitrogen percentages of the young plants were higher at the low than at the high temperature, except in groups 3 and 4 of table II, but these also showed the higher percentages at the lower temperature when calculated on the residual or fresh-weight basis.

6. pH values of the juice of the young plants were generally highest at the short day and at the low temperature and lowest at the long day and the high temperature. They were not affected appreciably by the differences in light intensity or soil reaction (tables II and III).

7. These various relations between chemical composition and environmental factors which appear in the data for the young plants were frequently not found in the older plants, which were sampled on different days as they reached the heading stage (tables IV and V).

8. The leaves of Turkey at the high temperature, where culm elongation was largely inhibited in this variety, had total carbohydrate and nitrogen values of the same order of magnitude as were found in the leaves of the normal heading plants of the low temperature. The only peculiarity noted

in the leaves of these non-heading plants was their unusually high glucose-sucrose ratios (table IV).

9. Such correlations as were found between carbohydrate and nitrogen values and fruitfulness occurred in the culm analyses only, and had no counterparts in the leaf analyses. The culms of the natural-day plants of Hard Federation, which at both temperatures produced relatively high yields of grain compared with those of the short and long days, were consistently characterized by the highest glucose contents and lowest nitrogen percentages, and the highest carbohydrate/nitrogen ratios. Both the natural- and long-day plants of Turkey at the low temperature gave high yields of grain, and their culms were likewise characterized by high carbohydrate/nitrogen ratios as compared with those of the sterile short-day plants (tables IV and V).

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DIVISION OF CEREAL CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

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PHYSIOLOGICAL ASPECTS OF THE EFFECT OF CONTINUOUS SOIL AERATION ON PLANT GROWTH[1]

W. F. LOEHWING

Introduction

The experiments here reported deal with the effects of continuous aeration of sand and soil cultures. They are the outgrowth of nutritional studies which suggested that the beneficial effect of certain fertilizers was ascribable to the production of larger root systems and increased absorption of nutrients. Since it was known that soil aeration would increase the bulk of root systems, experiments were undertaken to determine whether it was thereby possible to duplicate the results of certain fertilizer treatments and thus disclose that such nutrients acted indirectly by enlargement of the root system. The results of the aeration experiments are reported separately because they reveal significant effects of soil air on plant metabolism. Data from nutritional studies will appear elsewhere.

CANNON and CLEMENTS have reviewed the earlier literature on plant responses to soil aeration, and they have extended our knowledge of that subject by a comprehensive set of experiments of their own (**19, 24**). The existing data indicate that the growth of most roots depends upon free soil oxygen (**12, 39, 45**) although some roots can develop anaerobically (**16**). Anaerobic roots are characteristically devoid of root hairs (**16, 18, 41**) and consequently absorptive processes differ from those of typical roots (**26, 28**). Even those roots with low soil oxygen requirements, however, are readily injured by moderately high concentrations of soil carbon dioxide (**14, 15, 27, 37**). Relatively high oxygen tensions are needed to offset otherwise toxic carbon dioxide concentrations about roots (**17**).

Improper composition of soil air manifests itself in reduced, slow-growing root systems, inadequate absorption, short-lived, discolored foliage and delay or failure of reproductive processes (**1, 11, 19, 30, 34**). The symptomatic complex arising from impaired gas exchange of roots reflects a general reduction in rate and magnitude of normal absorptive and growth processes. The great bulk of existing evidence thus indicates that roots are sensitive to variations in soil air. It also suggests that experimental manipulation of soil atmosphere provides an effective means of studying the rôle of root systems and their effect on the metabolism of the plant as a whole. It must be noted, however, that the preponderance of existing data deals chiefly with minimal oxygen requirements, carbon dioxide tolerance, and a general description of gross anatomical changes induced as

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critical concentrations of both gases are approached. The general character of the results of earlier workers nevertheless suggests that root activity is influenced fully as much by soil air as by water and essential mineral nutrients. The data given here indicate some of the metabolic differences between aerated and unaerated plants.

Procedure

The experiment comprised a double series of pot cultures, one of which contained coarse quartz sand and the other an ordinary fertile field loam. Two-gallon, glazed earthenware jars were provided with a tubed $3'' \times 3'' \times 1'$ porous basswood block to distribute the air supply. Pots of the sand series were filled with 12 kg. of sand and each uniformly aerated with a continuous stream of air approximating 100 liters daily. The main air stream was scrubbed by bubbling through two 10-liter bottles partly filled with water. These bottles also served to regulate the pressure and to humidify the air, thus maintaining the soil moisture of aerated pots very nearly equal to that of controls. Use of dry air was found to cause injury largely through its evaporational effect even at rather low rates of aeration. Humidity and rate of flow were further regulated by interposing a smaller water bottle between each pot and the main air supply (**6**). Sand cultures were initially given 500 cc. of a neutralized Knop's nutrient solution which was gradually brought up to a total of 1500 cc. at the end of the first month. The soil solution varied between pH 7.4 and 7.0 during the course of the experiment.

All pots were maintained at approximately constant weight by watering as needed. A control series was similarly treated except that aeration was omitted. A second series employing 10 kg. of fertile loam was also aerated in the foregoing manner in order to determine the effect of difference in soil type. Dwarf sunflowers (Manus variety) were used in both sand and loam cultures as well as inoculated Ito San soy beans in loam.

Initial analyses were made four weeks after germination while the plants were still in the vegetative phase. The final analyses were made in seven to nine weeks when the plants were in flower but before fruits had appeared. Sand and soil were carefully washed out of the jars in order to harvest the entire plant with minimum injury to the root system. Plants were separated into tops and roots by cutting at the ground line. Roots were carefully freed of solid matter, rinsed in distilled water, blotted, and allowed to air-dry for 30 minutes. Fresh weights were recorded and tissues were then comminuted by hand or Nixtamal mill.

Material for carbohydrate and nitrogen analyses was preserved in 80 per cent. alcohol. Soluble carbohydrates were first hydrolyzed and the precipitated copper estimated as glucose according to official methods (**10**).

The insoluble residue was refluxed with hydrochloric acid and further analyzed as starch (**10**). Nitrogen of dry material and sap was determined by the Kjeldahl-Gunning method. Calcium was titrated as oxalate against permanganate. Magnesium and phosphorus were estimated colorimetrically as phosphates in a solution made up of ammonium molybdate, sodium sulphite, and hydroquinon. Potassium was precipitated as chloroplatinate and iron estimated colorimetrically in amyl alcohol as thiocyanate.

Tissue for sap analyses was placed in sealed vials, immediately frozen at $-60°$ C. in dry ice. Tissue was rapidly thawed prior to extraction. Sap was expressed hydraulically and filtered through a double cloth filter at 10,000 pounds' pressure. Conductivity, hydrion, buffer, and oxidation measurements were made at 25° C. and these determinations as well as the freezing-point depression were commenced immediately after extraction of the sap (**35**).

Sap samples were immediately cleared, acid-hydrolyzed, and analyzed for reducing components which are recorded as glucose. Oxidase activity was determined colorimetrically with an indophenol reagent freshly prepared for each determination as follows: 0.144 gm. alpha-naphthol was dissolved in 10 cc. of 95 per cent. alcohol; then 0.209 gm. p-phenylenediamine-hydrochloride was added and the whole made up to 250 cc. with water, and finally neutralized with anhydrous sodium carbonate. Exactly 1 cc. of sap was added to 50-cc. portions of the reagent and permitted to stand for one hour in a 400-cc. beaker. Controls were similarly prepared except that sap was omitted. After standing for one hour, 50 cc. of 95 per cent. alcohol were added to dissolve the indophenol; both control and test sample were compared colorimetrically with a color standard containing 0.353 gm. indophenol per liter. Test samples generally showed formation of larger amounts of indophenol, and this quantity minus that formed in the controls is expressed in the tables as grams of reagent oxidized per liter of sap per hour.

Hydrion and buffer capacity were measured potentiometrically with a calomel half-cell and hydrogen electrode. Two 4-cc. samples of fresh sap were used for the acid and the alkali buffer curve respectively and the pH recorded after successive additions of 0.5-cc. portions of N/40 acid and alkali respectively. Osmotic pressures were computed from depression of the freezing-point determined by the Beckman method (**35**).

Data on sunflowers

The analytical data for sunflowers in the sand and loam series (tables I, II) disclose a general increase in size and weight of roots as well as tops in the aerated cultures. Seedling development is accelerated by aeration and the precocity thus established is maintained well into the reproductive

TABLE I

ANALYSES OF SUNFLOWERS GROWN IN SAND

	Age 29 days				Age 50 days			
	Tops		Roots		Tops		Roots	
A	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
Fresh weight, gm.	4.15	3.03	3.30	1.97	8.90	7.10	7.50	5.23
Top/root ratio	2.09	2.51	.....	.....	2.98	3.41	.....	.....
Dry weight, per cent.	13.90	10.39	8.37	6.29	14.34	13.93	5.70	5.57
B	Percentage of dry weight							
	%	%	%	%	%	%	%	%
Total sugars	12.71	19.59	17.56	29.27	11.54	12.79	15.63	20.50
Starch	19.91	25.32	12.33	20.64	13.67	15.90	33.94	34.40
Nitrogen	1.48	2.75	1.13	1.68	1.94	2.00	1.10	1.13
Ash	7.81	9.19	31.77	21.82	7.71	7.36	12.02	19.26
Calcium	2.48	2.92	1.65	2.16	3.16	2.31	1.12	1.84
Potassium	0.76	0.67	2.07	3.80	0.54	0.46	0.87	1.14
Magnesium	0.36	0.25	0.45	1.27	0.47	0.80	0.73	0.87
Phosphorus	0.14	0.15	0.07	0.23	0.17	0.18	0.34	0.10
C	Actual weights of chemical constituents per plant							
Dry weight, gm.	0.58	0.31	0.28	0.12	1.28	0.99	0.43	0.29
Total sugars, gm.	0.07	0.06	0.05	0.04	0.15	0.13	0.07	0.06
Starch, gm.	0.12	0.08	0.03	0.03	0.18	0.16	0.15	0.10
Nitrogen, mg.	8.50	8.60	3.10	2.10	24.80	20.00	4.70	3.30
Ash, mg.	45.10	28.60	87.69	27.06	98.40	72.90	49.40	56.00
Calcium, mg.	14.00	9.00	4.56	2.68	40.30	23.90	3.25	5.35
Potassium, mg.	4.39	2.08	5.51	4.63	6.91	4.56	3.72	3.32
Magnesium, mg.	2.08	0.78	1.24	1.58	6.02	7.94	3.12	2.53
Phosphorus, mg.	0.81	0.47	0.19	0.29	2.18	1.79	1.46	0.03

TABLE I—(*Continued*)

	Age 50 days				Age 29 days			
	Tops		Roots		Tops		Roots	
	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
D	Grams per liter of expressed sap							
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Dry weight	56.12	39.82	21.28	22.33	63.18	43.38	19.46	12.58
Ash	5.26	6.48	2.37	3.97	5.16	5.75	2.96	4.46
Total sugars	2.07	2.99	15.15	11.70	16.88	10.85	8.20	6.40
Nitrogen	0.30	0.53	0.09	0.18	0.20	0.10	0.35	0.66
Indophenol	0.07	0.05	0.04	0.03	0.20	0.13	0.06	0.05
E	Osmotic pressure* and specific conductivity of sap at 25° C.							
Osmotic pressure	8.23	8.84	5.45	6.17	8.95	9.31	4.36	4.35
Conductivity	0.0116	0.0119	0.0094	0.0092	0.0116	0.0116	0.0092	0.0086
F	pH showing buffering action of 4 cc. of sap against fortieth normal reagent							
HCl n/40 3.0 cc.	4.46	4.46	3.93	3.99	4.61	4.50	3.70	.....
2.5 cc.	4.63	4.56	4.05	4.15	4.47	4.63	3.80	.....
2.0 cc.	4.81	4.71	4.19	4.31	4.96	4.77	4.00	3.80
1.5 cc.	5.09	4.92	4.34	4.36	5.24	4.98	4.19	4.02
1.0 cc.	5.51	5.22	4.53	4.66	5.66	5.26	4.40	4.27
0.5 cc.	6.17	5.62	4.84	4.95	6.25	5.71	4.77	4.65
pH of pure sap	7.09	6.71	5.81	5.27	7.06	6.45	5.74	5.54
KOH n/40 0.5 cc.	7.66	7.54	7.86	6.02	7.48	7.07	7.38	7.07
1.0 cc.	7.97	7.90	8.91	7.49	7.74	7.45	8.51	8.05
1.5 cc.	8.21	8.14	9.40	8.31	7.96	7.72	9.01	8.55
2.0 cc.	8.42	8.35	9.72	8.81	8.13	7.93	9.34	8.85
2.5 cc.	8.61	8.54	10.50	9.31	8.28	8.13	9.55	9.16
3.0 cc.	8.78	8.72	11.10	9.63	8.43	8.26	9.72	9.31

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.

TOP

TABLE II

ANALYSES OF SUNFLOWERS GROWN IN LOAM
PLANTS SIXTY DAYS OLD

	Tops		Roots		Tops		Roots	
	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
A					Actual weight in grams			
					gm.	*gm.*	*gm.*	*gm.*
Fresh weight, gm.	4.43	3.75	4.58	3.61	4.43	3.75	4.58	3.61
Top/root ratio	1.59	1.34	.....	.....	.....	.....	.....	.....
Dry weight	12.01%	11.18%	7.35%	8.49%	0.53	0.42	0.34	0.31
B	Percentage dry weight				Actual weight in milligrams			
	%	%	%	%	*mg.*	*mg.*	*mg.*	*mg.*
Total sugars	7.82	4.96	11.25	11.09	41.60	20.78	37.91	33.93
Starch	15.69	11.73	17.74	16.11	83.47	49.15	59.78	49.30
Nitrogen	5.61	3.79	2.08	1.82	29.75	15.88	7.01	5.57
Ash	17.46	15.75	8.57	9.60	92.89	65.99	28.88	29.38
Calcium	2.92	2.72	1.13	2.14	15.53	11.40	3.81	6.55
Potassium	1.90	1.15	0.87	1.26	10.11	4.82	2.93	3.85
Magnesium	0.48	0.47	0.92	1.19	2.55	1.97	3.10	3.64
Phosphorus	0.34	0.32	0.12	0.13	1.81	1.34	0.40	0.40
C	Grams per liter of sap				pH showing buffering action of sap†			
	gm.	*gm.*	*gm.*	*gm.*	4.93	4.86	4.87	4.81
Dry weight	47.84	45.68	25.33	33.10	5.09	5.00	4.93	4.92
Ash	12.68	11.20	5.38	7.04	5.30	5.17	5.01	5.01
Total sugars	6.90	5.16	5.48	11.03	5.57	5.40	5.15	5.05
Nitrogen	6.28	1.22	0.33	0.38	5.93	5.76	5.37	5.22
Indophenol	0.0506	0.0466	0.0714	0.0774	6.34	6.22	5.57	5.55
D	Sap osmotic pressure and conductivity*							
					6.97	6.78	5.88	5.49
Osmotic pressure	14.64	14.76	5.32	6.05	7.54	7.35	6.45	7.02
Conductivity	0.0290	0.0279	0.0112	0.0110	7.91	7.77	7.21	7.43
					8.20	8.13	7.69	7.66
					8.43	8.38	8.03	7.83
					8.63	8.59	8.35	7.96
					8.79	8.78	8.59	8.17

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.
† Readings as in table I, F.

phase. Although the controls are not invariably lower in percentage dry weight of each constituent, they are generally lower in corresponding absolute weights. Thus in the sand cultures (table I) the percentage ash in unaerated tops and roots is higher but the total weight of ash per plant is twice as great in young vegetative aerated plants. Differences in total ash tend to diminish with increasing age but aerated plants retain an appreciable excess even during senescence. Aerated plants absorb, transport, and utilize mineral nutrients more rapidly and efficiently than the controls if judged on the basis of rate of dry weight increase.

In connection with the essential inorganic materials, the small stature of the control plants explains the initially higher percentage of calcium despite the smaller total amount per plant in the sand cultures. There is an obvious tendency of calcium to accumulate in the roots of the controls in the maturation phases, both in sand and loam cultures. The absorption and internal distribution of magnesium is striking, occurring earlier and reaching the tops more rapidly in aerated young plants. This is evidenced by the fact that young aerated tops are much higher than the controls in magnesium while at the same time the unaerated roots run higher than aerated roots in percentage and total magnesium, both in sand and soil cultures (tables I, II). The original lag in magnesium intake by unaerated controls in sand was more than compensated, however, by rapid absorption during the seventh and eighth weeks. At the end of this time the total magnesium content of the mature controls in sand exceeded that of the aerated plants. The fact that this rapid intake of magnesium occurs late in the life of the controls and that it is coincident with a definite lag in potassium absorption may explain why the development of the sand controls fails to overtake that of the corresponding aerated plants (C, table I). Although less pronounced, similar results were observed in loam cultures. Acceleration in rate of magnesium intake is thus concomitant with a reduction in rate of potassium absorption.

Thus the initial percentage of potash is high in both aerated and unaerated plants but falls with increasing maturity, owing in part to the reason just given and also to the proportionately greater increase in dry weight over potash intake. Although percentage of potash in roots fluctuates, the aerated roots in sand are uniformly higher in absolute amounts of this element. Percentages of phosphorus are rather low in all young plants (**39, 40**) but increase significantly after the fifth week, especially in roots. Aerated plants not only run uniformly higher in total phosphorus but inaugurate reproduction earlier and maintain it longer than the controls.

The organic metabolites, namely, organic nitrogen, total sugars, and hydrolyzable polysaccharides, are uniformly higher in percentages but not

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.
† Readings as in table I, F.

in absolute amount in all unaerated sand plants. Aerated loam plants, however, were higher in both percentage and total amounts of these constituents. Although not given in the tables, supplemental analyses showed a tendency for crude fiber and total amino nitrogen to be greater in aerated plants, a condition suggestive of more rapid assimilation. Since high sugar content coupled with abundant nitrate nitrogen generally characterizes impaired protein metabolism, this was probably also the case in the above instance.

It will be noted, however, that the soluble constituents of the sap, as indicated under dry weight composition thereof, tend to be higher in the tops of older aerated plants in sand and loam. Judging from the data on freezing-point depression by expressed fluids, their mineral constituents are osmotically more effective than their organic solutes. The specific conductivity of the sap of aerated and control plants, however, does not vary as greatly as might be anticipated from the preceding data. Both the conductivity and the buffer capacity of the sap were difficult to correlate with the pH and the mineral content of the plants. Aerated sunflower tops in sand appear better buffered than controls against acid, but as they reach maturity these differences in acid buffering diminish greatly. Mature unaerated roots on the other hand possess a better buffer capacity against alkali than aerated cultures of similar age. The greater free acidity of sap from unaerated plants suggests a higher acid content than in aerated cultures (**21**).

Total nitrogen dissolved in expressed sap is generally low but shows a tendency to accumulate in unaerated roots of sunflowers grown in sand (D, table I). This fact again implies not only slower translocation of solutes but also impaired nitrogen metabolism in controls, especially in older plants. On the whole, the sap of the plants showing the best development was marked by a higher oxidation potential, shown by the greater amount of indophenol produced. On the other hand, the top-root ratio in terms of fresh weight of the best developed plants was consistently lower in aerated plants than for the poorer controls of the same series (A, tables I, II).

Data on soy beans

The soy bean loam cultures (table III) exhibit several points of contrast with sunflowers, owing to the difference in feeding habits of the two species. Aerated soy beans displayed more abundant root nodule formation, and these plants were larger. In contrast with sunflowers, calcium was more rapidly absorbed and translocated to the tops. Sunflowers and soy beans were comparable, however, in the accumulation of calcium in the roots of older unaerated plants.

TABLE III

ANALYSES OF SOY BEANS GROWN IN LOAM

	Age 27 days				Age 56 days			
	Tops		Roots		Tops		Roots	
A	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
Fresh weight, gm.	2.18	1.87	1.70	0.94	3.95	3.17	3.03	1.45
Top/root ratio	2.44	4.51	.....	.....	3.51	4.73	.....	.....
Dry weight, per cent.	13.29	12.76	6.99	5.65	17.69	15.24	6.56	7.00
B	Percentage dry weight							
	%	%	%	%	%	%	%	%
Total sugars	4.00	2.87	6.73	4.04	3.00	2.61	10.91	3.91
Starch	15.63	20.08	20.35	26.31	13.67	12.79	20.93	14.71
Nitrogen	4.92	3.41	4.82	2.20	3.45	3.98	3.62	3.11
Ash	9.98	11.25	22.00	32.25	9.15	11.87	8.94	13.16
Calcium	3.19	2.18	6.82	5.84	1.87	2.16	1.16	2.60
Potassium	1.70	1.04	3.65	4.51	1.39	1.44	0.78	1.56
Magnesium	0.24	0.23	1.42	3.18	0.41	0.43	0.81	1.07
Phosphorus	0.51	0.74	1.69	1.00	0.64	0.54	0.35	0.85
C	Actual weights of chemical constituents per plant							
Dry weight, gm.	0.29	0.24	0.12	0.05	0.70	0.48	0.20	0.10
Total sugars, mg.	11.80	6.80	8.00	2.12	21.00	12.60	21.80	3.90
Starch, mg.	45.00	47.90	24.40	13.90	95.60	61.70	47.80	15.00
Nitrogen, mg.	14.20	8.10	5.20	1.10	23.15	19.20	7.24	3.18
Ash, mg.	28.90	26.70	26.18	17.13	64.00	57.00	1.79	13.42
Calcium, mg.	9.25	5.21	1.00	3.10	13.09	10.43	2.30	2.65
Potassium, mg.	4.93	2.48	4.32	2.39	9.72	6.95	1.55	1.59
Magnesium, mg.	0.69	0.56	1.70	1.68	2.89	2.08	1.61	1.09
Phosphorus, mg.	1.47	1.77	2.02	0.53	4.48	2.61	0.70	0.87

TABLE III—(*Continued*)

ANALYSES OF SOY BEANS GROWN IN LOAM

	Age 27 days				Age 56 days			
	Tops		Roots		Tops		Roots	
	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated	Aerated	Unaerated
D	Grams per liter of expressed sap							
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Dry weight	46.22	42.02	18.84	19.22	64.96	59.47	16.48	19.40
Ash	14.40	14.99	3.12	5.03	12.60	12.05	3.92	6.65
Total sugars	2.23	2.63	5.16	4.11	5.30	4.43	1.69	1.73
Nitrogen	1.21	3.45	0.24	0.39	2.73	2.00	0.68	0.68
Indophenol	0.04	0.03	0.04	0.02	0.08	0.10	0.06	0.04
E	Osmotic pressure* and specific conductivity of sap at 25° C.							
Osmotic pressure	.....	.....	.....	.....	15.12	9.44	4.96	5.69
Conductivity	0.0158	0.0159	0.0162	0.0164	0.0331	0.0314	0.0108	0.0138
F	pH showing buffering action of 4 cc. of sap against fortieth normal reagent							
HCl n/40 3.0 cc.	.....	.....	.....	.....	7.06	6.48	9.03	8.04
2.5 cc.	.....	.....	.....	.....	5.07	5.08	3.21	4.16
2.0 cc.	.....	.....	.....	.....	5.18	5.20	3.45	4.37
1.5 cc.	.....	.....	.....	.....	5.31	5.32	3.74	4.64
1.0 cc.	.....	.....	.....	.....	5.45	5.45	4.06	5.00
0.5 cc.	.....	.....	.....	.....	5.61	5.58	4.46	5.45
					5.79	5.71	4.92	5.90
pH of pure sap	6.34	6.22	6.82	6.09				
KOH n/40 0.5 cc.	.....	.....	.....	.....	5.97	5.84	6.61	6.26
1.0 cc.	.....	.....	.....	.....	6.20	5.96	7.10	6.59
1.5 cc.	.....	.....	.....	.....	6.37	6.08	7.88	6.91
2.0 cc.	.....	.....	.....	.....	6.55	6.18	8.31	7.22
2.5 cc.	.....	.....	.....	.....	6.71	6.28	8.60	7.50
3.0 cc.	.....	.....	.....	.....	6.89	6.37	8.86	7.79

* Osmotic pressure given in atmospheres and specific conductivity in reciprocal ohms.

Aerated and control soy bean seedlings did not differ greatly in magnesium content, nor was there any acceleration in its absorption by older control plants as observed in sunflowers. Aerated soy beans were higher in total magnesium. Aeration seemed to favor potassium intake throughout, while appreciable potash accumulation occurred in older control roots. Aerated young plants were characterized by rapid entry of phosphorus into roots but slow movement into the tops. The tops of older aerated plants were higher in phosphorus than the roots, however, thus showing a later acceleration in rate of translocation in aerated plants. Total phosphorus was higher in the entire aerated plants.

Although percentage composition of total sugars, starch, and nitrogen in soy bean loam cultures (table III) varied slightly from corresponding sunflower tests (table I, II), the absolute weights were analogous, showing a tendency of aerated plants to contain greater absolute amounts of these materials.

Specific conductivity of sap (E, table III) was variable, no definite tendency being observable. Sap osmotic pressures and pH were uniformly higher in aerated young and old tops. Tissue fluids of young aerated and unaerated soy beans in loam were all rather weakly buffered, but differences developed with increasing age. Unaerated tops of older soy beans were better buffered against alkali (F, table III). Aerated roots were poorly buffered against both acid and alkali, with a steep gradient in the median portion of buffer curves. Sap dry weight, ash, total sugars, and nitrogen ran appreciably higher in aerated tops but lower in aerated roots (D, table III). Top-root ratios were distinctly smaller for most aerated plants, owing chiefly to the greater bulk of the root systems.

Discussion

The preceding data not only coincide with earlier work as far as the general formative effects of soil aeration are concerned, but also suggest (in part at least) the physico-chemical explanation of the structural changes noted in earlier studies by other investigators. In this investigation, not only were roots found to be sensitive to changes in soil oxygen and carbon dioxide (**9, 29, 36, 43**), but continued soil aeration was also found to induce marked changes in the structure of tops and roots (**8, 11, 14, 31, 32, 41**), and to alter entirely the development of the root habit (**13, 16, 33, 46**). Abundant aeration favored increased branching and bulk of the root system (**3, 4, 25, 40, 44**) as well as profuse hair production (**11, 16, 18**).

Such marked differences in root systems were correlated with definite effect on the tops. Aerated tops were more vigorous in appearance, with larger, dark green leaves and higher yield (**2, 5, 7, 8, 32, 34, 42**). In contrast with studies of certain other investigators (**5, 43**) seedlings in aerated

sand cultures germinated and grew much more rapidly. This initial advantage over controls was maintained through maturation, as manifested by earlier and more prolonged flowering as well as by heavier fruiting.

Other investigators have shown that proper root aeration is especially important in relation to the reproductive phase of growth (**1, 22, 30**), abundant soil oxygen being known to favor the setting and development of fruit. On the other hand, oxygen deficiency and carbon dioxide toxicity in the soil are known to induce premature abscission of reproductive structures (**1**).

The data here reported indicate on the whole that the combined effect of increased oxygen tension and lowered carbon dioxide content of the soil initially alters mineral metabolism in a marked degree, which in turn subsequently alters the basic organic syntheses of plants. It is probable that most plants normally encounter a gradual reduction of oxygen and increase of carbon dioxide in the soil, and that the soil atmosphere is thus normally an important factor in the developmental cycle. If, as suggested by PARKER (**38, 39**), the normal increments in soil carbon dioxide during plant growth do not induce appreciable alterations in mineral metabolism, this is probably due to the maintenance of a liberal supply of soil oxygen. Certainly the data indicate that soil aeration alters the nutrition and organic syntheses of plants.

As the preceding analytical data on the effects of soil aeration are reviewed, it is apparent that aerated plants were characterized by a much more rapid absorption and transport of mineral nutrients. This in turn was followed by acceleration in the rate of nitrogen and carbohydrate accumulation, especially in loam cultures.

The initial absorption of magnesium and potassium in aerated cultures was especially rapid. Although the intake of calcium and phosphorus was slower, it continued longer and in total amount was well above that in the corresponding unaerated controls. These facts correlate well with the larger size of the finely branched root system and the profusion of root hairs of aerated cultures. It would be surprising indeed if this great increase in absorptive area of aerated roots did not result in enhanced feeding power. The greater indophenol formation by the tissue fluids of aerated tops suggests that these plants also metabolize their organic synthates more rapidly than the controls.

In addition to these compositional differences, aerated plants differed structurally from the controls. The striking effect of aeration on tops was acceleration of growth in early stages, attributable to increased length of basal internodes rather than to an increase in the number. Contrast in internodal distance diminished noticeably above the median nodes, however, and there was little or no difference in size of internodes near the

tips. Elongation continued in the controls after the aerated plants had started to flower. This response tended to reduce size differences when mature plants of both groups were compared. In fact, in the older soy bean plants in loam the percentage root dry weight of controls showed a tendency to exceed that of aerated plants. In 60-day old sunflowers continued dry weight increase of control roots also resulted in a lower top-root ratio, a response opposite to that found in the other instances. The physiological effect of aeration on tops thus appeared to be acceleration in development with earlier maturation rather than prolonged development.

With reference to the structural characteristics of aerated roots, it may be said that they were more fibrous, longer, and more highly branched. The root hair zone of aerated plants, although having more roots per unit surface, was less extensive longitudinally and the hairs were shorter lived, apparently because of the more rapid elongation of aerated roots. Internally the vascular tissues just above the root hair zone were not as definitely differentiated in aerated roots as in the controls. Other internal structural comparisons were difficult because of differences in size of aerated and control roots of similar age, but in general the walls of root cells in aerated plants were heavier, and such roots showed a slight tendency for intercellular spaces to increase as plants matured (7). Microchemical tests for starch and hemicelluloses were more pronounced in cells of aerated roots near the root hair zone.

Aeration of soil about the roots of aquatic angiosperms is known to produce formative responses similar to those described here. DEAN (25), working in this laboratory, has found that root systems of submerged aquatics, such as *Typha, Sagittaria,* and *Hibiscus,* increase greatly in size in aerated sand, clay, and muck. Roots in aerated soils were longer and more highly differentiated, as shown by heavy lignification of the new primary roots in many cases. In the main, unaerated roots were fibrous but less numerous than the corresponding fibrous elements of aerated root systems. Submerged (but not subterranean) water roots of *Typha* and *Sagittaria* were more numerous and profusely branched in unaerated soils. In every case larger tops were associated with more extensive root systems.

Perhaps the outstanding contrast between soil aeration of aquatic and terrestial plants is the effect of soil type. The structural characteristics of aquatics do not vary so greatly with soil differences as they do in land plants. Formative differences between aerated and unaerated sunflowers and soy beans grown in loam were never so marked as those in sand. Unaerated controls of soy bean, sunflower, and several other species not here discussed essentially overtook the aerated plants in size of tops in the maturation phase.

In conclusion it must be pointed out that the nature of the plant response

to soil aeration varies considerably from one species to another, and also varies with the degree of aeration. Sunflowers in pot cultures, although responding favorably to aeration rates less than 10 liters per kilogram of soil per day, were distinctly retarded in growth when this rate was doubled (**45**). Soy beans, were not so easily injured as sunflowers by rapid aeration, however, a contrast especially apparent in warm moist weather (**20**). In fact, tolerance of higher rates of aeration is increased as temperature rises.

It is interesting to note that similar responses have been recorded by other investigators for plants grown in solution cultures. Buckwheat, for example, is not benefited by rates of aeration known to be favorable to other plants (**23, 27**), and may in fact be injured if dry weight is taken as a criterion. Similar responses have recently been reported for aerated solution cultures of tomatoes. These also flowered and fruited later than the controls (**23**). The scanty data of other investigators on the reduced development of the root systems of plants injured by excessive aeration confirm those of the writer. Aeration uniformly increases the fibrous character of roots, but when over-aeration causes injury, the size of such injured roots is considerably diminished.

Summary

1. Aerated sunflower and soy bean plants grown in sand and loam differed from unaerated controls in the following ways:

(a) Taller in size and heavier in weight as a result of early rapid growth.

(b) Larger in root system, more fibrous, and more highly branched; root cells heavier walled and higher in reserve carbohydrates.

(c) More rapid nutrient absorption as shown by higher content of ash, calcium, potassium, and phosphorus per plant in terms of absolute weight of entire plants.

(d) Higher in total weight per plant of crude fiber, starch, total sugars, and nitrogen.

(e) More alkaline expressed sap in tops and roots; tops had a higher buffer capacity but the roots a lower buffer capacity against alkali and acid.

(f) Smaller top-root ratios in terms of fresh weight than in controls.

2. The difference between sand and loam cultures is expressed by a tendency of percentage composition of certain components to run higher in controls, even though absolute amounts of such constituents are consistently higher in aerated plants. Sap hydrion values were generally higher in plants grown on sand.

3. Moderate rates of continuous soil aeration with moist air increased size and growth rate of plants, but very rapid aeration had the opposite effect.

4. Species differed in their tolerance of soil aeration. The threshold of injury by rapid aeration is higher for soy beans than for sunflowers, especially when dry air is used.

UNIVERSITY OF IOWA
IOWA CITY, IOWA

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POTENTIALLY UNLIMITED GROWTH OF EXCISED TOMATO ROOT TIPS IN A LIQUID MEDIUM

PHILIP R. WHITE

(WITH THREE FIGURES)

Introductory

In the cultivation of isolated tissues, whether plant or animal, it is necessary to distinguish between *growth* at the expense of the culture medium and *survival,* in which the medium plays only an inert or, at most, a secondary rôle. In the first case the nutrient is itself the limiting factor in determining the degree and to some extent the character of the increment obtained. Given a satisfactory nutrient, adequate replacement of exhausted nutrient, and adequate removal of excretory products, growth should continue indefinitely. In the second case increment may also take place for a time at the expense of materials reabsorbed from the older portions of the original explant, the medium acting essentially as an inert substratum only. As these reabsorbed materials are exhausted, the culture will gradually succumb, no matter how often the medium is renewed. An extended period of *growth* ordinarily implies the maintenance of a high level of metabolism. *Survival,* on the other hand, is ordinarily dependent on *minimal* metabolic rates, and is hence prolonged by depressant conditions such, for example, as moderate cold, low oxygen supply, etc.

The literature of the field of plant tissue cultures contains accounts of many experiments which clearly fall in the latter category. Reference need only be made to the work of HABERLANDT, BOBILIOFF-PREISSER, BÖRGER, CZECH, KUNKEL, PRÁT, ÚLEHLA, WHITE, *et al.* This literature has been reviewed more extensively elsewhere (**16**). All of these authors obtained *survival* of isolated tissues under various conditions, often for months, but no true growth. Perhaps the most striking example of this type of result is that of BAILEY and ZIRKLE (**1**) who, using sections of cambium from woody plants immersed in such non-nutrient media as paraffin oil, were able to maintain active protoplasmic cyclosis for many weeks. This is obviously a phenomenon of retarded metabolism only, a mere slowing down of the process of dying.

The experiments of KOTTE (**9**), ROBBINS (**12, 13**), ROBBINS and MANEVAL (**14, 15**), MALYSCHEV (**10**), and WHITE (**17–21**) are less easily classified. In many cultures of these workers active increment did take place, and in the work last cited the nature of the nutrient obviously did contribute quite largely in determining the extent and character of growth obtained. Only comparatively short culture periods were employed, however, and the absolute amounts of increment were small. It therefore is possible that this

increment may also have been dependent in part on materials carried over in the original fragment. The experiments of ROBBINS and MANEVAL (**15**), which represent the most extended periods (five months) and the greatest numbers of passages (ten) reported to date, strongly suggest that this was the case, since the observed mean growth rates decreased rather consistently from about 7.8 mm. per day in the first passage to 0.86 mm. per day in the tenth passage, after which the cultures were lost. Thus the growth rate at any passage bore an inverse relationship to the total amount of tissue formed up to that passage. This would appear to indicate that the rate of increment might have been determined by the concentration of some unknown material furnished to the culture from the tissues of the original fragment and gradually diluted in the multiplication of these tissues. If this be true, these cultures also were cases of *survival*, though for long periods. There are nowhere in the literature of plant tissue cultures accounts of any experiments which can with certainty be said to fall within the first category outlined above.

In view of this uncertainty and of the importance of the concept of *complete nutrients* in the use of a tissue culture method in the study of physiological and pathological problems, it has seemed desirable to determine whether or not a plant tissue can *grow* at the sole expense of a nutrient, without the *quantity* of material contributed by the original fragment entering as a limiting factor. A positive answer to this question will require that such a tissue be maintained in a state of active increase over a long enough period of time and through a sufficient number of passages to insure a dilution of all materials contained in the original fragment beyond the point where they could possibly be effective in determining growth rates. Such has been the purpose of the experiments presented in this paper.

Materials and methods

The present work represents essentially a repetition and extension of the earlier root tip cultures of ROBBINS (**9, 10**) and ROBBINS and MANEVAL (**11, 12**). Tomato was chosen as a test organism, since it was planned to use the tissue culture technique later in the study of certain diseases to which this plant is susceptible. It is assumed that wheat (**18, 19**) would have given similar results.

The methods of culture were approximately those outlined elsewhere (**18, 19**). The seed to be used were removed under aseptic conditions from clean healthy fruit of the Bonny Best variety, grown under greenhouse conditions. They were usually germinated on sterile filter paper. As soon as the roots had attained a length of 5 mm., and some days before the cotyledons appeared, they were severed and placed in 125-ml. Erlenmeyer flasks containing 50 ml. of nutrient each. A single root was placed in each flask.

The nutrient medium used was approximately the same as that developed in previous work (**19**), with some slight modifications. It contained the following salts at the partial concentrations indicated:

SALT	MILLIMOLS
$Ca(NO_3)_2$	0.60
$MgSO_4$	0.30
KNO_3	0.80
KCl	0.87
KH_2PO_4	0.09
$Fe_2(SO_4)_3$	0.006

To this were added 2 per cent. by weight of sucrose, which for tomato was found experimentally to be superior to the dextrose used in previous experiments (**18, 19**), and the filtered extract of 0.01 per cent. by weight of dried brewers' yeast (**18**).

In making up the nutrient, the sequence of mixing the various ingredients appears to be important. The method found most satisfactory was to proceed as follows: The first four salts listed above, $Ca(NO_3)_2$, $MgSO_4$, KNO_3, and KCl were dissolved together in one flask. The KH_2PO_4 and $Fe_2(SO_4)_3$ were each dissolved separately. These were then mixed in a quantity of distilled water such as to make one half of the final volume of nutrient desired, and set aside until the yeast was ready, usually about two hours. The sucrose was dissolved in a quantity of water somewhat less than half the volume of the final nutrient and also set aside. The yeast was boiled for half an hour in 100 to 200 times its volume of distilled water, and filtered twice under suction, using no. 597 Schleicher and Schüll (or no. 1 Whatman) paper. The salts, sugar, and yeast extract were then mixed together, made up to volume, and distributed to the culture flasks, 50 ml. per flask. These were autoclaved immediately at 1 atm. pressure for 20 minutes. They were ready for use as soon as cool.

All cultures made previous to October 5, 1933, were placed on a bench in diffuse sunlight in a potting shed subject during the summer months to the temperature fluctuations of the outdoor atmosphere. On October 5th the cultures were transferred to a northeast laboratory where better temperature control was available. Here also they received diffuse sunlight during the daylight hours. The method of making the original cultures has been outlined above. Subcultures were made by cutting the roots into fragments each bearing one or more growing points. Each cutting was placed in a flask of fresh nutrient.

Experimental results

ROBBINS's experiments suggested the existence of some limiting factor present in the original fragment of tissue, diluted as this tissue multiplied,

not renewed under the cultural conditions employed, and hence fixing a limit to the amount of growth obtainable in such cultures. In examining further into the probability of the existence of such a factor and hence into the probable limits to which such excised root tips may be grown *in vitro*, data from two clones of cultures, designated as B and C, will be considered. Each clone was derived from a single root tip and was therefore genetically homogeneous.

In the preparation of clone B aseptic seeds of Bonny Best tomato were placed in a series of flasks. This series was started on November 2, 1932. Five days later the seed had begun to sprout and on November 11 the seedling roots were severed. The ages given in the following discussion are measured from this date. The apices were retained in the flasks, the seeds and seedlings being discarded. The fragments retained were about 10 mm. long. After some days the most vigorous examples were selected for further culturing. By November 21, that is 10 days after excision, the particular root under consideration had attained a length of 32 mm., with one branch. The primary root soon stopped growing, and the growing point turned brown, as often happened, especially in the earlier cultures. The lateral, however, continued to grow and to branch further. On November 30 the culture was transferred to a fresh nutrient but without cutting back.

A completely satisfactory experimental complex had not been developed at this time. Rather than risk the loss of the culture by transferring again to a nutrient of uncertain value, the flask was set aside while further experiments on methods were being made. The culture was left undisturbed for 145 days until April 24, 1933. At the end of this time the root had attained a total length, including branches, of 4564 mm., with 181 growing points. It was then removed and divided into 50 pieces, each piece being placed in a fresh flask. Twenty of the 50 subcultures were so made as to contain about 8 mm. of apical meristem each, without any mature tissue. These 20 grew much more satisfactorily than did the apices on larger masses of tissue,

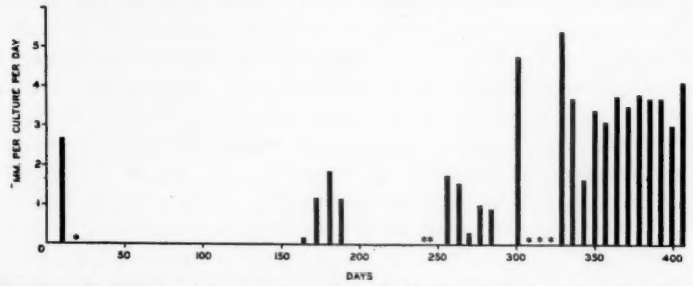

FIG. 1. Increment rates of clone B during 406 days, 29 passages. The asterisks represent passages in which no measurements were taken.

the old tissue apparently exerting a certain inhibitory action. All subsequent cultures were therefore made from these smaller pieces. The cultures grew quite consistently, though not very rapidly (fig. 1), remaining at the bottom of the flasks. On May 2, 11, and 18, subcultures were made, all but about 5 to 10 mm. of meristematic tissue being discarded at each transfer. They were then set aside again until July 10, an interval of 53 days. By this time they were brown, without active growing points, and apparently dead. Nevertheless, upon transfer to fresh nutrient, they resumed growth and put out fresh branches so that by July 14 it was possible to again sever the growing points, discarding all but about 5 mm. of each. Subcultures were made on July 25, August 1, 8, and 15. Up to this time the cultures had remained at the bottom of the flasks, growing only slowly, but branching with great profusion.

The low increment rate and high branching rate of those cultures which remain at the bottom of the flasks appear to be correlated with low respiration rate, but whether the fact of sinking to the bottom is itself the cause or an effect of some unknown condition remains to be determined. The two conditions are concomitant. Of the 50 cultures made on August 15, three floated to the top of the nutrient. These were all derived from no. 17 of the previous (12th) passage, and were designated as nos. 4, 5, and 6. Coincident with the floating of these individuals to the top of the nutrient, their increment rates quadrupled as compared with their previous values, the branching rate showing a corresponding fall. No. 4, for unknown reasons, ceased to grow after a week's time. No. 6 became contaminated with bacteria and had to be discarded. No. 5 remained in good condition and ultimately became the foundation for the entire clone. Although transfers on September 1 (14th passage) included material from 6 examples of the previous passage, all except the descendants of this one actively growing culture were eliminated by selection in the next two weekly passages. Detailed daily records were begun on September 28 in the 18th passage, when the culture was 322 days old, and were continued until it had reached the age of one year. In passages after that date the lengths of the roots at the beginning and end of each passage were recorded, giving a measure of the increment rates for each week, but without the daily detail. Records of the clone were discontinued at the end of the 28th passage, at an age of 405 days, but the clone is, at the time of preparation of this paper, alive and growing normally at an age of over 500 days.

Figure 1 shows the results of observations throughout 406 days of the life of this clone. Following the elimination of the slower growing members, the last 16 passages show a markedly *increased* mean growth rate over that of the previous 9 months. Figure 2 is of a single subculture photographed at the age of 367 days (passage, 12 days) and again 4 days later.

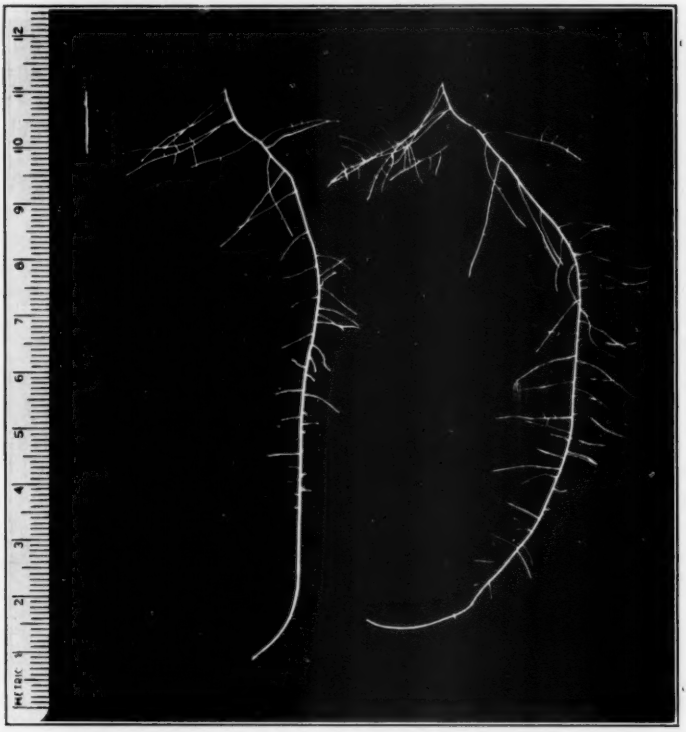

FIG. 2. A typical subculture of clone B, passage 23. The figure at the upper left shows the size of the fragment at the beginning of the passage, aged 355 days. At the center 12 days later, and at the right the same culture after 4 days more.

The increase both in length and in number of branches is evident, and represents what may be considered as approximately normal growth for tomato root tips. The fact that an isolated root tip can continue to maintain approximately normal activity at the end of a year's time in an artificial environment, is good presumptive evidence of its capacity to grow indefinitely under such conditions.

The records kept of clone C are somewhat more complete and continuous. Seeds of Bonny Best tomato were germinated aseptically on filter paper and on March 1, 1933, 25 severed root tips were placed in flasks of a nutrient (not the standard described above) which later proved only partially satisfactory. A single one grew, and this only poorly. On March 13 this one root was transferred to a more satisfactory nutrient in which it grew actively though somewhat unevenly. On March 29 it was divided into

16 subcultures and on April 21 two of these were selected and again divided into 42 and 24 pieces respectively. Neither the nutrient solution nor the culture method was at this time entirely satisfactory and experiments were conducted aiming at their improvement, using the cultures of this clone as experimental material. In the next 7 passages, therefore, the transfer periods and nutrients used were not uniform. With the 11th passage, at an age of 127 days, the nutrient solution and method of preparation outlined at the beginning of this paper were established as standard, and a period of one week chosen for the length of passage. The actual data must therefore be divided into two groups, the first embracing the first 10 passages, the second all passages subsequent to the 10th. The latter group should be much more nearly homogeneous and more reliable as a basis for drawing conclusions. Since at the date of preparation of this paper the clone is still being maintained, so that the data cannot be considered as a completed whole, a period covering 52 passages has been arbitrarily chosen for consideration in detail. The second homogeneous group of data, from the 11th to the 52d passage, thus embraces 294 days under standard conditions. These data are summarized in table I.

TABLE I

LINEAR INCREMENTS AND NUMBERS OF BRANCHES PRODUCED FROM A SINGLE ROOT DURING 52 PASSAGES

PASSAGES	TOTAL OBSERVED INCREMENT	TOTAL OBSERVED NUMBER OF BRANCHES	NUMBER OF PASSAGES	NUMBER OF DAYS RECORDED	MEAN PASSAGE LENGTH	MEAN BRANCHING RATE PER DAY	MEAN INCREMENT RATE PER DAY
	mm.						*mm.*
1–10	14,319	3,044	10	127(–52)*	13	0.385	1.91*
11–17	18,379	3,505	7	49	7	0.716	3.75
18–24	18,759	3,756	7	49	7	0.766	3.83
25–31	26,769	6,582	7	49	7	1.303	5.48
32–38	26,158	6,657	7	49	7	1.383	5.33
39–45	23,084	5,143	7	49	7	1.064	4.71
46–52	27,379	6,912	7	49	7	1.091	5.59
Total ...	154,847	35,399	52	421	.....	0.958	4.37

* Detailed linear increment measurements were not made in the first 2 passages totaling 52 days. For that reason, although the first 10 passages totaled 127 days, the calculation of mean increment rate is based on 127–52, or 75 days only.

In 52 passages (421 days) the clone made a total measured increment, exclusive of all branches, of 154,847 mm. To this are to be added the increments of 35,399 branches 1 mm. or more in length. Since these average at least 5 mm. each, their total length would be about 175,000 mm. The total observed increment for 52 passages was, therefore, about 430 meters, or about 1300 feet. This was all derived from a single growing point approximately 10 mm. long. That a single root-tip can thus be maintained and made to multiply at approximately normal rates through 52 passages, is again strong presumptive evidence that it can be maintained indefinitely.

The details of the data further support this general presumption. In figure 3, A, the mean linear increment rates (exclusive of branches) are plotted as ordinates against the passage numbers as abscissas, while in figure 3, B, the mean numbers of branches formed per culture per passage are similarly shown. Each point on the curve of linear increments represents the mean value obtained from daily observations on 100 cultures over individual passages of 3 to 28 days. Since all passages after the 10th were uniformly 7 days in length, each point for the last 42 passages thus represents the mean of 700 observations. Each point on the curve of branching rates represents the mean number of branches on 100 cultures.

That portion of each curve representing the first ten experimental passages need not be considered in detail. The remainder, covering 42 passages under standard conditions, may be divided into five parts. Passages 10–14 show a steady rise both in increment rate and branching rate believed to have been brought about by gradual selection within the clone. Passages 14–17 are marked by an equally regular and very sharp fall in both indices. The cause of this was found to be lead poisoning from a faultily repaired water still. After this was corrected, passages 17–26 showed a quite regular increase, broken somewhat at the 19th and 20th passages, when transfers were made by one of the writer's colleagues,[1] and at the 24th and 25th passages when drastic culling, carried out in an effort to eliminate a troublesome brown discolored condition present in some cultures[2] resulted in discarding many cultures, thus lowering the average increments as estimated on the basis of the initial 100. Passages 26–39 then showed comparatively uniform growth indices, the mean linear increments oscillating quite regularly between extremes of 4.5 and 6.8 mm. per culture per day. The branching indices fluctuated somewhat more widely and erratically. A second

[1] It is a pleasure to acknowledge the help of Dr. L. O. KUNKEL who undertook the task of making the transfers, and of Dr. E. L. SPENCER who made the necessary measurements during this period.

[2] This condition was of such a nature as to suggest the presence of a bacterial infection. Attempts to find any organism, either by microscopic examination or by plating on various standard bacteriological culture media all failed, however. The incidence of this condition was greatly reduced by repeated selection, but its cause is still unknown.

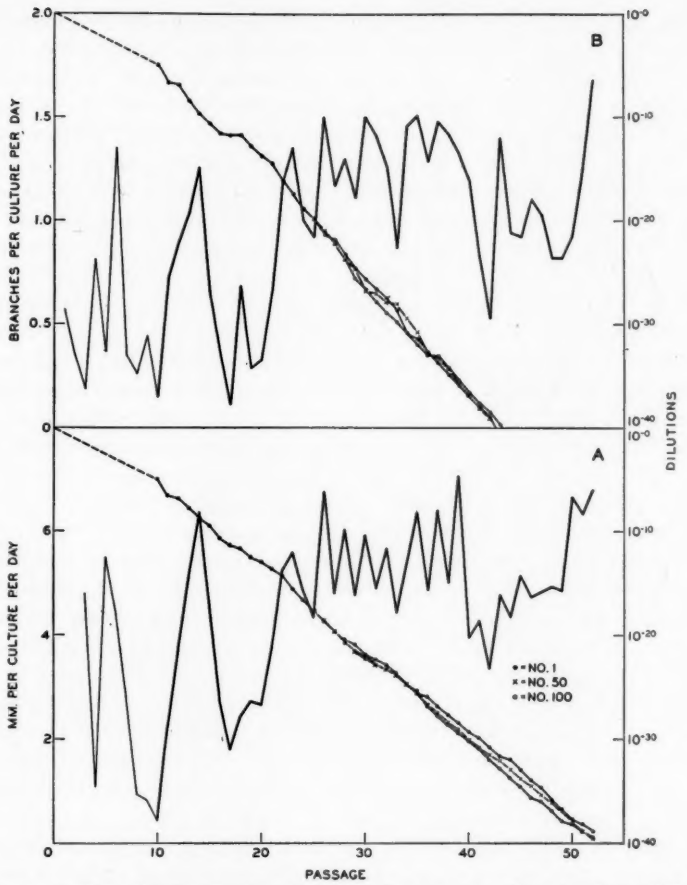

FIG. 3. A. Mean increment rates of clone C, in mm. per culture per day, plotted by passages. The corresponding dilutions of any material from the initial fragment distributed uniformly to all tissues formed are plotted for three individual cultures for comparison. B. Branching rates of clone C in numbers of branches formed per culture per day. The corresponding dilutions of any material from the initial fragment distributed uniformly to all growing points are plotted for the three examples as in A.

sharp drop in indices occurred in the 40th passage. The reason for this has not yet been satisfactorily traced, but the fact that both indices have subsequently recovered makes it clear that the drop was due to some uncontrolled external factor. The character of growth obtained at the end of the experiment was quite normal, the roots being clean, white, straight, with normal branching habit, and growing at rates which were not markedly sub-

normal. Individual cases of roots growing 30 to 40 mm. per day were fairly frequent. The factors which affect growth rates will be discussed more extensively elsewhere. The important fact brought out by these curves is that, unlike ROBBINS's cultures, the changes in growth rates can in no way be correlated with number of passages and can therefore *not* be interpreted as resulting from a time or dilution factor. This is perhaps even more clearly brought out by a comparison of clones B and C from the point of view of the relative effects of age and previous treatment. Clone B was approximately 200 days old in the 6th passage, while clone C had a similar age in the 21st passage. The linear indices at this age were for clone B 0.7, for clone C 3.7 mm. per culture per day. On the other hand, 134 days later the *simultaneous* indices were: clone B (18th passage) 5.3, clone C (23d passage) 5.6 mm. per culture per day. It is obvious from these figures that neither age nor member of passages, but simultaneity (and hence identity in environmental conditions) was the crucial factor in determining growth rates. It is the precise details of the environmental complex rather than any characteristics, other than genetic ones, inherent in the material of the explant, which determine the rates of growth at any moment.

Two strains of cultures have then been maintained for more than a year, one through 52 passages and the other through 30 passages. The measured tissue increment has been many thousands of times the original value. The mean growth rates have been clearly independent of origin, age, number of passages, previous treatment, etc., but closely dependent on the detailed variants in the artificial environmental complex. Hence it may be concluded with reasonable safety that the cultures have ceased to be dependent on any material factors carried over from the parent plant. The environmental complex used is therefore adequate for all requirements for growth of such tissues. The cultures have been shown to be capable of unlimited growth in such an environment.

The conclusion just drawn, that isolated root tips can be grown indefinitely under the experimental complex employed, may at first seem acceptable without further proof. Nevertheless, the data in the form presented above still leave a logical doubt as to its validity. The dilution of materials derived from the original explant, calculated as the ratio between initial tissue volume and total observed increment, has, even in clone C, been only of the order of $10/150{,}000 = 6 \times 10^{-5}$. It is well known that many materials: hormones, enzymes, viruses, etc., are effective at much greater dilutions than this. Even some of the essential nutrient elements, such as boron, become toxic above concentrations of the order of molar $\times 10^{-5}$. Copper, zinc, iodine, etc., are effective in even smaller quantities. It is hence quite conceivable that even at a dilution of 10^{-5} there may still remain sufficient quantities of limiting materials carried over from the original frag-

ment to be effective, although complete elimination of these materials would prevent further growth. In order to remove this doubt, it is therefore necessary, as stated in the introduction to this paper, to answer the question: "Can it be shown that the greatest possible crucial dilution for all conceivable materials of the original fragment must have been surpassed in the experiments under consideration?" This question has certainly not been answered in the foregoing section.

The experimental data do, however, furnish an affirmative answer if treated in a manner slightly different from that used above. If the pedigrees of three representative cultures of the last (52d) passage, nos. 1, 50, and 100, are traced back, it is found that all three were derived from culture no. 99 of passage 28, and further back from culture no. 57 of passage 11 when the standard environmental complex was introduced. Their histories from passage 11 to passage 28, through 17 passages, were thus identical. The detailed records show that this initial culture of the 11th passage, no. 57, was, when started, 7 mm. long, growing during the passage to a length of 36 mm. In passage 12 it was initially 11 mm. long, growing to 65 mm., the corresponding figures being 14 and 67 mm. for passage 13. The dilution of any material derived from this initial culture and uniformly distributed to all tissue formed therefrom during these three passages would thus be $7/36 \times 11/65 \times 14/67$, or 7×10^{-3}. The dilution obtained in this way for the 17 passages in which the history of the three examples was identical was about 3×10^{-12}, and the final dilutions for the three at the end of the 42 passages under standard conditions (52d passage) were 1.5×10^{-34}, 4.5×10^{-36}, and 5.3×10^{-36}. If, instead of considering the increment in volume of tissue, the numbers of growing points formed are taken as a measure of the dilution, the values obtained are still higher. During passages 11 to 15 the numbers of branches formed were 4, 8, 3, 22, and 19, giving a dilution of 2.5×10^{-5}. The final dilutions based on numbers of branches formed would then be 1.9×10^{-45}, 2.3×10^{-46}, and 1.6×10^{-45}. None of these figures include any of the dilution occurring during the first 10 experimental passages, which is roughly estimated to have been of the order of 10^{-5}. It is thus evident that whether this hypothetical limiting substance be considered as distributed to all tissues or only to growing points, its "dilution" at the end of the 52 experimental passages would be not less than approximately of the order of 10^{-40}.

Now, the pieces of tissue employed in starting each subculture weighed on the average about 2 mg. or 2×10^{-3} gm. fresh weight. A molecule of water has a mass of approximately 3×10^{-23} gm. If the root tips used as subcultures be considered to be made up of water only, such a piece would thus contain approximately $2 \times 10^{-3}/3 \times 10^{-23} = 0.67 \times 10^{20}$ molecules. If this be diluted as has been shown to have been the case in the cultures under

consideration to a value of the order of 10^{-40} times its original value, it is evident that there could still be only $10^{20} \times 10^{-40} = 10^{-20}$ part of one molecule of the material of the original root tip present in each culture of the 52d passage. This result obviously represents a *reductio ad absurdum*, and since the increment rate (fig. 3, A) at the end of the 52d passage has been equalled in only two previous passages, nos. 26 and 39, and the branching rate (fig. 3, B) is higher than at any previous time in the entire 400 odd days, the idea of any limiting factor in the form of a "hormone" or other material furnished to the culture from the parent plant is untenable. The results of the experiments of ROBBINS and MANEVAL, which seemed to indicate the existence of such a factor, must be explained in some other way.

This conclusion, of course, in no way affects the possibility of hormones being manufactured by the root itself. But if such exist they are not factors limiting the potential amount of growth obtainable in culture, and hence of no particular interest in the problem at hand. Moreover, so long as all the observed facts in a given series of experiments are, as in the present case, explainable without resorting to such hypotheses, and since the existence of such hormones can be demonstrated only by the behavior of cultures in their absence—a condition not demonstrably obtained in these cultures—the introduction of such hypotheses appears to the author to be an unnecessary encumbrance.

It appears, then, to have been demonstrated that the nutrient and the environmental complex employed in these experiments are adequate to sustain normal growth of such root tips indefinitely.

Discussion

It is important to note that in thus isolating a plant root from the parent plant and placing it in an artificial nutrient which is demonstrably adequate for its continued growth, a method is presented for an analysis of those factors by which the parent plant under natural conditions nourishes and controls this organ. It has been generally supposed, though without experimental demonstration, that the reciprocal relationship by which the aerial portions of the plant are supplied with salts and water by the roots and these in turn are supplied with the products of photosynthesis by the tops, was one necessary for the continued well being of both. The exact form in which these photosynthetic products were furnished to the roots has remained in doubt. It has also been doubtful just where the seat of synthesis of proteinaceous materials lay, whether in roots or aerial organs, and whether this process might not likewise be dependent on photosynthesis.

The present investigation has brought answers to some of these questions and furnishes a means not previously available of obtaining data pertinent to others. The interdependence of top and root is *not* a necessary one, at

least as far as the root is concerned, since these roots have shown unmistakably that they can be grown indefinitely without ever, even as seedlings, having been under the influence of tops. The artificial medium has provided adequate substitutes for whatever products the tops ordinarily send down to the roots. The photosynthetic product has been replaced by sucrose. The salts supplied have been those used for entire plants and, as was to be expected, their assimilation is quite independent of green tissues. The proteins required have evidently been manufactured in the roots, from the elements supplied in the nutrient. There remains, aside from possible traces of impurities, only one factor in the environmental complex supplied which is recognized as an unknown, namely, the extract of dried yeast. Whether this is itself replaceable by more easily analyzable materials remains to be seen.

It is interesting to compare the nutrient used in these experiments which has proved adequate to supply all the needs of a plant tissue, with the corresponding nutrients used for similar cultures of animal tissues. A great part of the work on the latter material has followed the methods introduced by HARRISON (**8**) and developed by CARREL. These methods will, therefore, be considered for comparison. In the work outlined above, the nutrient has consisted of three categories of materials: (1) the salt solution, (2) the carbohydrate, and (3) the organic material added as yeast extract. Of these, the exact constitution of the first two, representing 99.99 per cent. of the mass of the nutrient, is, aside from possible impurities, precisely known and can be varied at will. Only one component, the yeast extract, representing 0.01 per cent. of the entire mass, is of unknown constitution.

The nutrient generally used by CARREL (see CARREL, EBELING, FISCHER, ERDMAN, *et al.*), on the other hand, contains *no salt solution* and *no carbohydrate*. In its usual form it consists of two components, chicken serum making up from 50 to 70 per cent., and embryonic juice from 30 to 50 per cent., of the mass of the nutrient. A satisfactory modification used by EBELING (**5**) contained fibrinogen (prepared according to MELLANBY, **11**) 12.5 per cent., chicken serum 37.5 per cent., and embryonic juice 50 per cent. Dry weights are not usually given for these constituents, so that they cannot be directly compared with the constituents of the plant culture nutrient. But it is clear that the animal nutrient differs radically from the plant nutrient in being made up entirely of complex organic materials. DREW (**4**) has replaced the serum of CARREL'S medium with a salt solution consisting of NaCl, $CaCl_2$, KCl, $NaHCO_3$, $MgHPO_4$, and $CaH_2(PO_4)_2$, and has replaced the embryonic juice with autolyzed tissue extract or tumor extract, with satisfactory results. In this case, the known elements are somewhat greater, but still represent less than half of the mass of the entire nutrient. It has also been shown that the embryonic juice can be replaced

by a fibrin digest prepared either by the action of pepsin (BAKER and CARREL, **2**) or by controlled bacterial digestion (FISCHER and DEMUTH, **7**). These represent, to a certain extent, attempts to reduce the medium to simple components which can be adequately analyzed. But even in the case of DREW'S salt solution it should be noted that, although the cations Na, Ca, K, and Mg are supplied, there has been no attempt to supply a balanced *anion* portion, the SO_4 and NO_3 ions being entirely omitted, and the PO_4 and CO_3 ions introduced only because of their buffer action. The other salt solutions occasionally used in such work: RINGER'S, TYRODE'S, etc., are, like DREW'S solution, made up of chlorides only, with PO_4 or CO_3 or both added only as buffers. Such unbalanced salt solutions, as has been experimentally demonstrated elsewhere (**19**), would be entirely inadequate for plant tissues.

It is, of course, difficult if not impossible to determine the exact constitution of such complex, essentially organic nutrients, while the determination of the constitution of the simple, essentially inorganic nutrient used in these plant cultures is comparatively simple. It is equally impossible to vary the concentrations of the active constituents, except in a very gross way. So long as such complexes as blood serum and embryo juice make up the greater part, or all, of the nutrient, the only nutrient variants available to the experimenter are varying proportions of these complexes. CARREL, BAKER, and others have made analyses which indicate that the proteoses play an important rôle in the activity of embryo juice, but these are again complex substances of undetermined constitution (FISCHER, 6). The complex nutrients have been retained because they fulfilled the two basic requirements of (1) complete adequacy for maintenance of unlimited growth, and (2) simplicity and duplicability of preparation. Perhaps if such a natural nutrient had been found in the early development of plant tissue cultures, it too would have been retained and used without further search. The latter field has, however, had a history quite the reverse of that of the animal field. Every attempt to find a natural culture medium comparable to that of CARREL, prepared from embryo juices, pressed saps, sieve tube exudates, endosperm fluids, digests, etc., has consistently failed with, in most cases, marked evidence of toxicity (see HABERLANDT, THIELMANN, ÚLEHLA, PRÁT, ROBBINS, MAYER, and others). Because of this failure, the workers in the plant field have been forced to adopt the reverse method, of starting with a simple medium of known constitution and, by a study of the individual constituents of this medium in their effects on growth and by the addition of new constituents, to build up an increasingly adequate, and at the same time precisely known, substratum and general complex for the maintenance of the cultures. The result has been a cultural complex which, as shown in the present paper, possesses both of the essential char-

acteristics of the animal complex, complete adequacy for the maintenance of unlimited growth, and simplicity and duplicability of preparation, and which in addition, as shown elsewhere (**18, 19**), possesses a flexibility far greater than that of the complex used in the older field.

Summary and conclusions

Tomato root tips, excised, and grown in a simple liquid medium of known constitution, have been maintained for over a year, and through 52 passages, in continuous active growth as measured by increase in number of growing points and total volume of tissue. One isolation has produced *in vitro* approximately 35,000 growing points and more than 400,000 mm. of tissue, from an initial fragment 10 mm. long. Calculations based on averages from approximately 40,000 separate observations on this clone give the theoretical dilution of any material of the original fragment as well beyond 10^{-40}, hence beyond the limits of molecular dilution. The entire material of most, if not all, of the ultimate cultures is therefore necessarily derived from the nutrient. This nutrient is thus demonstrated to be adequate for *all* growth requirements of such tissue. The growth rates have shown no consistent tendency to fall off, there being, on the contrary, a consistent increase in the early passages from 1.5 mm. per culture per day in the 1st to 6.8 mm. in the 26th passage, the corresponding value at the 52d passage being also 6.8 mm. per culture per day. It is thus proved that tomato root tips are capable of maintaining an independent, active, apparently normal existence *in vitro* for potentially unlimited periods of time, under such an artificial environmental complex as has been described here.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

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SALT CONCENTRATION AND REVERSIBILITY OF ICE-FORMATION AS RELATED TO THE HARDINESS OF WINTER WHEAT[1]

S. T. DEXTER[2]

(WITH SIX FIGURES)

Although the conditions of ice-formation in the tissues of plants have been the subject of numerous investigations, in the literature there appears to be no description of detailed quantitative experiments regarding the reversibility of the process of ice-formation. In several papers the reversibility or irreversibility of the process is implied. Thus, MÜLLER-THURGAU (**14**) showed that the freezing point of tissue was lower on the first freeze than on refreezing, which suggests irreversibility; NEWTON and GORTNER (**15**) specifically warned against refreezing the sample of expressed sap in determining "bound water." To the contrary, irreversibility might be implied by the technique used by STEINMETZ (**20**), who froze samples of alfalfa roots at an extremely low temperature, before expressing the sap to be used in "bound water" studies.

The question of reversibility of ice-formation in non-living colloidal systems has been repeatedly investigated. (*Cf.* JONES and GORTNER (**11**) for recent literature.) Such systems are sometimes indicated as "elastic gels" when reversible, and "non-elastic" gels when irreversible.

That the capacity for reversibility might well be significant in a consideration of winter-hardiness is brought out by casual observation of frozen plants as they thaw out. One may observe that, hardy and tender alike, the plants immediately after thawing have a water-soaked appearance; in the case of hardy plants, however, this water is reabsorbed in the course of a relatively short time and the plants regain their former appearance. If a temperature too cold for the survival of the hardy plants is used, this reabsorption does not occur.

When plant tissues freeze, the formation of ice removes water from the sap, thereby concentrating the solutes in the unfrozen water until the cryohydric point is reached. A study of the concentration of the minerals under such conditions is of interest, since "salting out" of colloids, often irreversibly, is a matter of common occurrence.

Experimental material

To examine this problem in an experimental way the following procedure was followed. Field plats of four varieties of winter wheat were seeded

[1] Contribution from the plant physiology laboratories, University Farm, University of Minnesota.

[2] National Research Council Fellow.

at the usual time and in the usual manner by the Agronomy Department of the University of Minnesota. No attempt was made to select varieties of peculiar varietal hardiness. However, the varieties in the probable order of their cold-resistance were as follows: (1) Minturki-Marquis hybrid (very hardy); (2) Minturki (very hardy); (3) Kanred (medium hardy); (4) Blackhull (medium hardy). These plats were not replicated and no pretense is made that, in their development, the typical hardiness of the varieties was necessarily developed. When samples were obtained at intervals throughout the autumn, every effort was made to obtain typical samples from the plats; however, on the last date (November 21) the ground was deeply frozen and covered with snow, and the samples were selected from small continuous areas which had been covered with paper after the ground froze but before the snow fell. The samples were prepared by removing the roots and leaf blades, together with any dried external leaf-sheaths. This material was discarded. The crowns were quickly washed by shaking with distilled water in a large beaker. In order to bring all of the samples to a comparable degree of dryness, the washed crowns were placed upon cellucotton pads and covered with a damp towel. In the course of an hour or two, the samples became almost free from surface moisture. During the process of weighing the samples, the crowns were further dried with cellucotton. After weighing, the samples were immediately wrapped in tin-foil. Percentage of dry matter was determined in similar samples by killing in an oven at 100° C. and drying *in vacuo* at 75° C.

Experimental methods

Determination of ice-formation

While several methods have been repeatedly used for the quantitative determination of ice-formation, it seemed that, for the experiment planned, the use of the calorimetric method would be most suitable. Recent work with this method is described in papers by Sayre (**19**), St. John (**21**), Meyer (**13**), and Robinson (**17**). Briefly, the method depends upon the heat of fusion of the ice formed; obviously, if, in a given sample, more ice is formed on one occasion than on the next, more heat will be required to melt the sample when more ice is formed. While several formulae have been devised, differing only slightly from one another, the following is used throughout this work.

$$\text{Ice, grams} = \frac{T_c\ (\text{T. C. cal.}) - T_s\ (\text{T. C. sample})}{80 - 0.5\ T_f}$$

where, T_c is the temperature change of the calorimeter, as indicated by the Beckman thermometer; T. C. cal. is the thermal capacity of the calorimeter, with 300 cc. of water; T_s is the temperature change of the sample, from the

frozen to the final melted condition; T. C. sample is the thermal capacity of the sample; and T_f is the temperature at which the sample was frozen, with the sign changed from negative to positive.

T. C. sample was calculated as follows: T. C. sample = grams water in sample + 0.35 (gm. dry matter) + 0.1 cal. The specific heat of the dry material in the sample was estimated at 0.35 calories per gm.; the 0.1 calorie represents the thermal capacity of the tin-foil used.

The calorimeter was a large silvered Dewar flask, heavily wrapped in cellucotton and stoppered with a thick cork stopper. Through the cork, a curved glass stirring rod and a Beckman thermometer projected into the flask. The top of the apparatus was further protected from air currents by a sheet of rubber through slits in which the stirrer and thermometer extended. 300 cc. of distilled water somewhat above room temperature were placed in the flask. To determine the thermal capacity of the calorimeter, stirring rod and thermometer, pieces of ice, frozen from distilled water, were dropped into the apparatus. Pieces with approximately the same heat of fusion as the samples were used, *i.e.*, about 15 gm. The average of 5 or 6 very closely agreeing replicates was used to compute the thermal capacity of the calorimeter.

Several methods have been suggested for the freezing of the samples and their transference to the calorimeter vessel. The following, which combines features of the methods of several investigators, was finally adopted. The samples of wheat crowns, weighing 20 gm., were wrapped in tin-foil and placed in tapered copper tubes. These tubes were placed in the freezing bath. If unwrapped samples were placed in tubes, it was found that considerable condensation of water vapor occurred on the inside of the tube. This would have introduced a very considerable error. Furthermore, the samples were found to freeze so firmly to the sides of the tube, if not wrapped, that is was impossible to remove them quantitatively or rapidly. When the large samples are wrapped in tin-foil, however, they melt with undue slowness, and the temperature equilibrium is markedly delayed. This delay is highly undesirable, for there is an inevitable, though slow, drift in the temperature of the calorimeter, due to the temperature difference between it and the air of the room. This drift may amount to as much as one one-hundredth of a degree a minute even when all precaution is taken to minimize it. To insure rapid melting in the calorimeter, the wrapped sample was removed from the copper tube after freezing thoroughly and the wrapper slit lengthwise, several times. The sample was handled with a paper pad kept in the cold room with the freezing bath, for that purpose. After slitting, the sample was replaced in the tube and freezing continued. With such treatment, thermal equilibrium was attained in the calorimeter in from 2 to 3 minutes, if the samples were vigorously stirred.

Large baths of alcohol-ice slush were prepared and kept in a cold room held at approximately the temperature desired. Alcohol or water was added to the baths to bring them to within one-tenth of a degree of the temperature required. The baths were readily kept at the desired temperature by frequent adjustments. All thermometers were calibrated in terms of one accurate thermometer. The Beckman thermometer was so adjusted that it read 21.4° C. below the actual temperature. It was read to the nearest hundredth of a degree.

Previous to freezing, the wrapped samples were stored at 2° C. overnight in the copper tubes. Duplicate samples of a single variety were placed in the freezing bath at the same time; after an interval of 30 minutes, a pair of duplicates of another variety was added and so forth. At the end of two hours of freezing, one sample was used for calorimetric measurements; the other, in its copper tube, was placed in a water bath at 2° C. where it remained for two hours. It was then refrozen for two hours at precisely the same temperature, when it was similarly used in the calorimeter. Thus, ice formation was determined in one sample after a single freezing treatment and in the duplicate after freezing, thawing and refreezing.

Freezing injury

After the sample and the calorimeter had reached thermal equilibrium, the sample with the 300 cc. of water was emptied into a beaker, the tin-foil removed, and the beaker, covered with a watch-glass, was transferred to a cold room held at 2° C. After 22 hours in this room, the electrical conductivity of the liquid was determined at 25° C. This gave an estimate of the freezing injury, according to the method of Dexter *et al.* (**5, 6**).

Total extractable salts

The sample, with the liquid, was heated to boiling and promptly placed again in the cold room. At the end of 24 hours the electrical conductivity of the liquid was again measured, in order to determine the total extractable electrolytes in the sample. In several cases electrical conductivity measurements were made after longer intervals, up to six days; but the increase in extracted electrolytes was slight. Since relatively insoluble materials might go into solution on long standing, or since autolysis might occur, setting free electrolytes, the conductivity of the solution after 24 hours of extraction is considered to indicate the total amount of extractable salts in the samples.

Discussion of methods

From the preceding description, it is evident that the following values were determined for each of the four varieties of wheat on the various dates:

1. Grams dry matter in the sample.
2. Grams water in the sample, by difference.
3. Grams ice frozen on the first freezing treatment, and water unfrozen, by difference.
4. Grams ice frozen on the second (identical) freeze, after two hours thawing, and water unfrozen, by difference.
5. Electrolytes extracted in 22 hours after freezing injury, following single and double freezing treatment.
6. Total electrolytes extractable in 24 hours, following killing of the samples by boiling.

Determinations of the freezing points of the expressed saps were made on October 21 and November 22. The freezing points for the four varieties ranged from $-1.0°$ to $-1.25°$ on both dates, with considerable difference between duplicates. The sap of the hardier varieties appeared to have a slightly greater concentration of solutes as measured in this way.

Since the temperatures used in this experiment, $-7°$ C. and $-13°$ C., are above the cryohydric point of the salts commonly found in plants, it is likely that precipitation of the salts did not occur during freezing, and that the salts remained in solution in the unfrozen water. If this is the case, we are able to compute the average concentration of salts in the unfrozen water, *i.e.*, the concentration of salts to which the protoplasm is subjected on freezing. While it is rather commonly agreed that a certain amount of water is associated with the colloids in the plant, the opinion has been recently expressed in several papers that this water is probably partly if not wholly available for the solution of salts and sugars in the sap (BRIGGS (**3**), HILL (**10**), and GORTNER (**8**). Thus, while the addition of gum arabic, gelatin, or similar material to a sugar solution undoubtedly increases the freezing point depression, the water calculated as "bound" has not lost its colligative properties. Evidently if the colloid behaves in a manner similar to that of ordinary solutes, the amount of water bound by the colloid will depend upon the desiccating forces to which it is subjected, *i.e.*, less water will be "bound" at $-13°$ C. than at $-7°$ C. Furthermore, binding capacity is influenced by the nature of the solutes present. BRIGGS (**2**) found that the presence of sodium chloride and potassium chloride decreased the water binding power of gum arabic, and that calcium ions lowered the hydration capacity of gelatin more than did sodium ions.

It is not my purpose to discuss the status of the "bound" water problem. A full literature citation and review is given by GORTNER (**8**). No attempt has been made in these experiments to determine the bound water in the samples; an attempt has been made, rather, to determine the amount and reversibility of ice-formation at temperatures sufficiently low to differentiate by freezing injury, the hardiness condition of the plants.

TABLE I

ICE-FORMATION AND PERCENTAGE DRY MATTER ON VARIOUS DATES

VARIETY	−7° C. SERIES				−13° C. SERIES				
	FROZEN ONCE		FROZEN TWICE		FROZEN ONCE		FROZEN TWICE		
	ICE	UNFROZEN WATER	ICE	UNFROZEN WATER	ICE	UNFROZEN WATER	ICE	UNFROZEN WATER	DRY MATTER
	October 5								
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
Minturki-Marquis	12.00	5.52	14.19	3.33					12.4
Minturki	11.57	5.80	14.62	2.75					13.65
Kanred	13.25	4.39	15.66	1.98					11.8
Blackhull	12.48	5.18	15.00	2.66					11.7
	October 19 and 20								
Minturki-Marquis	8.97	7.67	11.81	4.81	14.27	2.37	14.79	1.85	16.8
Minturki	11.24	5.06	12.38	3.94	14.25	2.05	14.28	2.02	18.5
Kanred	11.33	5.31	13.00	3.64	14.63	2.01	15.19	1.45	16.8
Blackhull	12.32	4.46	13.54	3.24	15.27	1.51	15.25	1.53	16.1
	November 1 and 2								
Minturki-Marquis	9.50	6.42	11.39	4.53	13.31	2.61	13.49	2.43	20.4
Minturki	10.47	5.76	10.85	5.38	13.82	2.41	13.96	2.27	18.85
Kanred	10.99	5.46	12.17	4.28	14.25	2.20	14.95	1.50	17.75
Blackhull	11.31	5.17	12.11	4.37	14.48	2.00	15.00	1.58	17.6
	November 22 and 23								
Minturki-Marquis	9.91	5.94	11.32	4.52	13.27	2.58	13.46	2.39	20.75
Minturki	10.82	4.76	11.22	4.36	13.34	2.24	13.37	2.21	22.1
Kanred	10.84	5.18	12.03	4.00	13.92	2.10	13.96	2.06	19.9
Blackhull	10.87	5.24	11.40	4.71	13.96	2.15	14.20	1.91	19.45

Experimental results

Table I shows the percentage of dry matter, the amount of ice frozen, and of water unfrozen on first and second freezing treatment of each sample of the four varieties of winter wheat on several dates. Minturki-Marquis and Minturki are known to be hardier than Kanred and Blackhull. Table II summarizes table I. The following facts appear:

TABLE II

SUMMARY OF TABLE I

DATE	−7° C. SERIES			−13° C. SERIES		
	TOTAL UNFROZEN WATER			TOTAL UNFROZEN WATER		
	FROZEN ONCE	FROZEN TWICE	RATIO	FROZEN ONCE	FROZEN TWICE	RATIO
	gm.	*gm.*		*gm.*	*gm.*	
October 5	20.89	10.72	0.513	.....	.....	.....
October 19	22.50	15.65	0.695	7.94	6.85	0.853
November 1	22.81	18.56	0.814	9.22	7.78	0.845
November 22	21.12	17.59	0.833	9.07	8.57	0.945

DATE	TOTAL DRY MATTER	UNFROZEN WATER PER GRAM OF DRY MATTER			
		−7° ONCE	−7° TWICE	−13° ONCE	−13° TWICE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
October 5	9.81	2.13	1.09	.....	.....
October 19	13.64	1.65	1.15	0.582	0.502
November 1	14.94	1.53	1.24	0.617	0.521
November 22	16.44	1.29	1.06	0.552	0.521

1. There is invariably more water frozen on the second freezing than on the first, but this tendency becomes less marked as the hardening process proceeds. Figure 1 shows this in graphic form. The amount of water left unfrozen on the second freezing treatment is 51 per cent. of that left unfrozen after one freezing treatment on October 5 (−7° C.) As the plants harden, the values approach each other more closely, until on November 22 the ratio is 83 per cent. This indicates that the ice-formation becomes more nearly reversible as the plants harden in the field, and that the period allowed for reabsorption of water after freezing is ineffectual in the case of the tender plants.

2. There is almost invariably more water left unfrozen in the hardy varieties than in the less hardy ones.

3. The amount of water left unfrozen per gram of dry matter is greater,

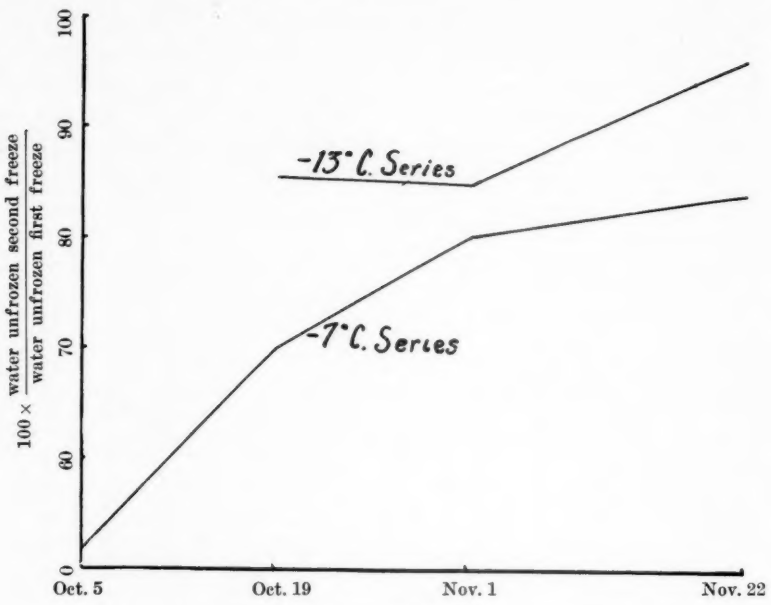

FIG. 1. Ratios of $\frac{\text{unfrozen water, second freeze}}{\text{unfrozen water, first freeze}}$ are shown on the vertical axis. This gives a measure of the reversibility of water withdrawal to form ice. On October 5 there was about twice as much water left unfrozen after the first freeze (– 7° C.) as after the second. As the season advanced the amounts became more nearly equal.

on the first freeze, – 7° C., when the plants are in the unhardened condition than when they are hardened (MEYER, **13**). On the second freeze, – 7° C., there is little difference in hydration between the hardened and unhardened condition. The same may be said of the – 13° C. freezing treatments. There appears to be little evidence that there is a greater hydration of the colloids in the hardened state; but it would appear that the hydration is more stable. Some indication of a reason for this greater stability will be brought out later, when mineral concentrations are considered.

Table III shows the amount of water, dry matter, and extractable electrolytes in 20-gm. samples of wheat crowns of four varieties taken from the field on different dates. These samples, with the exception of those on the last date were never frozen in the field. The value for extractable salt is the specific conductivity ($K \times 10^5$, 25° C.) of the solution into which the electrolytes diffused. These conductivity values are average of duplicates, single and double freeze, – 7° C. which rarely varied more than one per cent. from the mean. The last two columns of the table show the computed

TABLE III

TOTAL WATER, DRY MATTER, AND EXTRACTABLE ELECTROLYTES ($K \times 10^5$, 25° C.) IN SAMPLES ON VARIOUS DATES

DATE	VARIETY	WATER	DRY MATTER	EXTRACTABLE SALTS	SALT PER GRAM WATER	SALT PER GRAM DRY MATTER
		gm.	*gm.*	$K \times 10^5$	$K \times 10^5$	$K \times 10^5$
October 5	Minturki-Marquis	17.52	2.48	109.0	6.21	44.0
"	Minturki	17.27	2.73	116.0	6.67	42.5
"	Kanred	17.64	2.36	120.7	6.83	51.2
"	Blackhull	17.66	2.34	115.6	6.54	49.3
	Average			112.8	6.56	46.75
October 19	Minturki-Marquis	16.64	3.36	84.4	5.07	25.1
"	Minturki	16.30	3.70	90.2	5.52	24.4
"	Kanred	16.64	3.36	100.5	6.03	29.9
"	Blackhull	16.78	3.22	91.0	5.42	28.3
	Average			91.5	5.51	26.42
November 1	Minturki-Marquis	15.92	4.08	72.0	4.53	17.65
"	Minturki	16.23	3.77	79.5	4.89	21.10
"	Kanred	16.45	3.55	81.6	4.98	22.95
"	Blackhull	16.48	3.52	78.6	4.77	22.35
	Average			77.9	4.79	21.01
November 22	Minturki-Marquis	15.85	4.15	64.3	4.05	15.50
"	Minturki	15.58	4.42	70.1	4.51	15.85
"	Kanred	16.03	3.97	72.4	4.52	18.29
"	Blackhull	16.11	3.89	72.2	4.48	18.55
	Average			69.8	4.39	17.05

amount of soluble salt in each gram of water or of dry matter, respectively.

From the table, the following facts appear:

1. The water in the samples decreased as the season advanced and the dry matter increased. This has been the finding of workers, generally, in this field.

2. The total extractable electrolytes decreased regularly with each of the four varieties.

3. The salt per gram of water in the plant decreased as the season advanced, in spite of the fact that the amount of water decreased. That is, the concentration of salt in the sap, assuming all water to be free for solvent purposes, decreased regularly (figure 2).

4. The soluble salt per gram of dry matter decreased very markedly as hardening proceeded (figure 3). Furthermore, the amount of extractable salt per gram of dry matter appears to be related to the hardiness of the

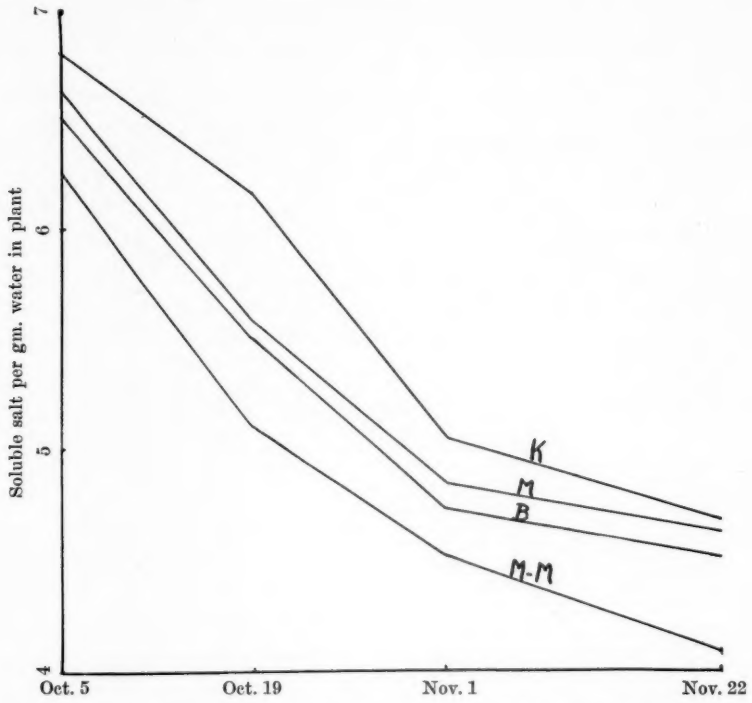

FIG. 2. Total extractable electrolytes from each sample (expressed as specific conductivity $\times 10^5$) was divided by the grams of water in that sample to give the values on the vertical axis. This gives the relative concentrations of salts in the cell sap on the dates shown. A progressive decrease in salt concentration is shown in all four varieties. M-M = Minturki-Marquis; M = Minturki; K = Kanred; B = Blackhull.

variety, and is generally lower in the hardy ones (ROSA (**18**), CHANDLER (**4**), GORKE (**7**)).

Since the amount of unfrozen water was determined in each sample together with the amount of soluble salt, one may readily calculate the concentration of salt per gram of unfrozen water. Furthermore, measurements were taken of the exosmosis of electrolytes following freezing, to give an estimate of freezing injury. Table IV presents these values. The "salt per gram of unfrozen water" was calculated by dividing "extractable salt" (table III) by "grams unfrozen water" (second freeze) (table I). The freezing-exosmosis conductivity values are the average of the same duplicates, except for the values given for October 5, where the value obtained for the sample frozen twice was used. On this date, the sample frozen twice was

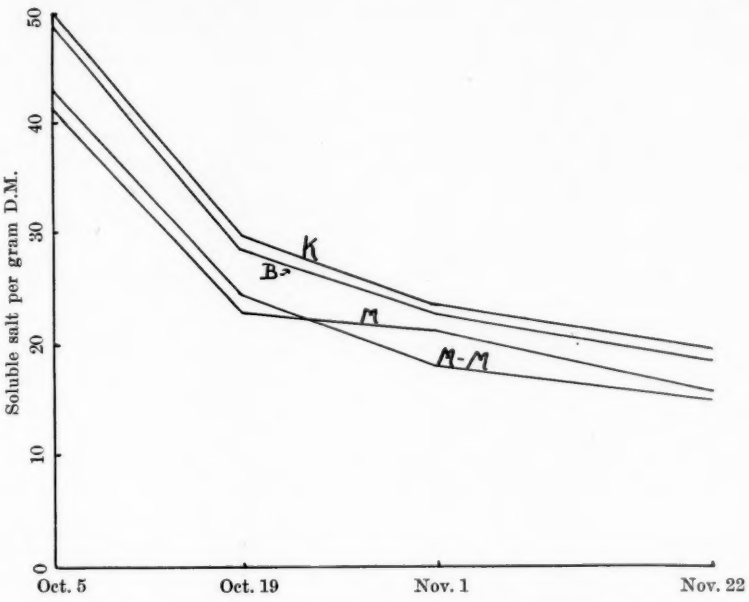

FIG. 3. Progressive change in ratio of $\frac{\text{soluble electrolytes}}{\text{grams dry matter}}$ in the four varieties of winter wheat. M-M(Minturki-Marquis hybrid) and M(Minturki) are hardier than K(Kanred) and B(Blackhull). The hardier varieties have in general a lower ratio of soluble salts to dry matter. In all varieties the ratio decreases greatly as the season advances and the plants increase in hardiness.

materially more injured than the sample frozen once only, and thus the conductivity value used represents the true injury more closely than would the average.

From table IV the following facts appear:

1. In a given variety, as hardening proceeded, the concentration of salts in the unfrozen water decreased to about one-third the value found in the early fall (−7° C. freeze) (figure 4).

2. The concentration of salts in the unfrozen water is regularly greater in the varieties known to be hardier by agronomic experience. Almost invariably the freezing-exosmosis values for frost injury are in the same order as the values for salt concentration in the unfrozen water. Figure 5 shows this in a graphic way.

From these results, it would appear that freezing injury may well be closely correlated with the concentration of salts in the unfrozen water. This theory is, in a way, by no means new. GORKE (7), HARVEY (9), and

TABLE IV

CONCENTRATION OF SALTS PER GRAM OF WATER LEFT UNFROZEN, TOGETHER WITH FREEZING-EXOSMOSIS VALUES OF INJURY FROM FREEZING

VARIETY	OCTOBER 5		OCTOBER 19		NOVEMBER 1		NOVEMBER 22	
	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY	SALT PER GRAM UNFROZEN WATER	FREEZING-EXOSMOSIS SPECIFIC CONDUCTIVITY
				– 7° C. series				
	gm.	$K \times 10^5$	*gm.*	$K \times 10^5$	*gm.*	$K \times 10^5$	*gm.*	$K \times 10^5$
Minturki-Marquis	32.7	67.7	17.5	29.6	15.88	23.3	14.37	17.9
Minturki	42.2	72.5	23.0	42.6	16.25	31.7	16.02	20.1
Kanred	60.9	91.8	27.8	48.0	19.02	33.9	18.10	21.65
Blackhull	43.4	84.3	28.0	48.6	17.88	31.0	15.36	22.9
				– 13° C. series				
Minturki-Marquis			47.7	63.0	29.7	47.2	29.3	42.2
Minturki			46.0	74.1	37.2	55.9	33.2	43.2
Kanred			71.7	85.2	58.6	63.2	37.3	46.9
Blackhull			61.3	75.5	51.2	57.8	42.0	48.2

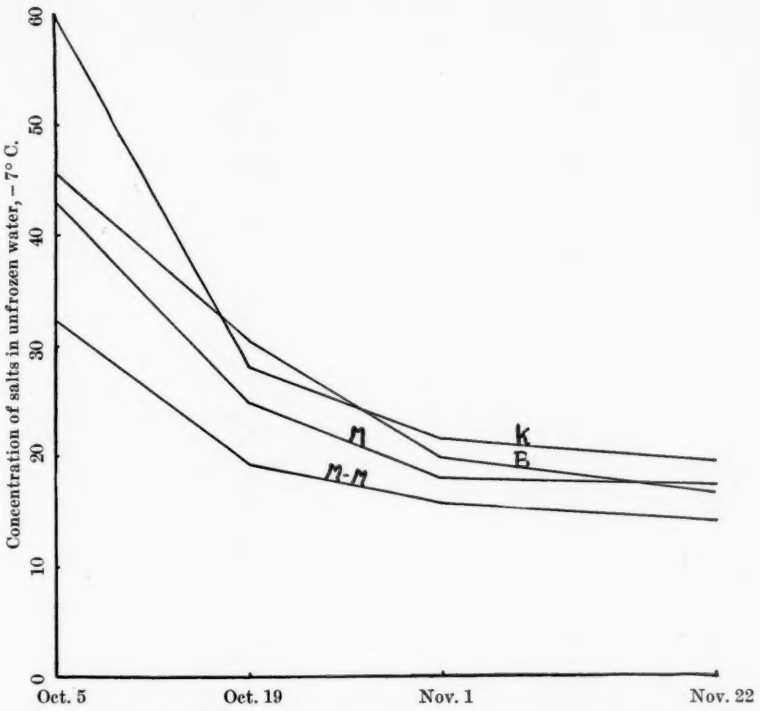

FIG. 4. Total extractable electrolytes of the sample (expressed as specific conductivity $\times 10^5$) have been divided by the values for grams-unfrozen-water to give the values on the vertical axis (– 7° C. series). Each curve shows the change in the electrolyte concentration in the unfrozen sap for a given variety as the season advanced. M-M(Minturki-Marquis) and M(Minturki) are hardier than K(Kanred) and B(Blackhull).

others have suggested that the injury to the protoplasm was due to the increase in salt concentration on freezing. The addition of sugars to plant saps or the accumulation of sugars within the plant has been repeatedly shown to favor winter hardiness (ÄKERMAN, **1**) and to prevent coagulation of proteins in the frozen expressed juice (NEWTON and BROWN, **16**). In either case, one evident effect of the added sugars would be correspondingly to decrease the concentration of salts in the sap at the freezing temperature used. The removal of electrolytes by diffusion, or by other methods, has not been shown, however, to cause any "protection" of the plant or the plant sap (NEWTON and BROWN, **16**, DEXTER, unpublished data).

That the winter wheat plant becomes relatively low in soluble salts and

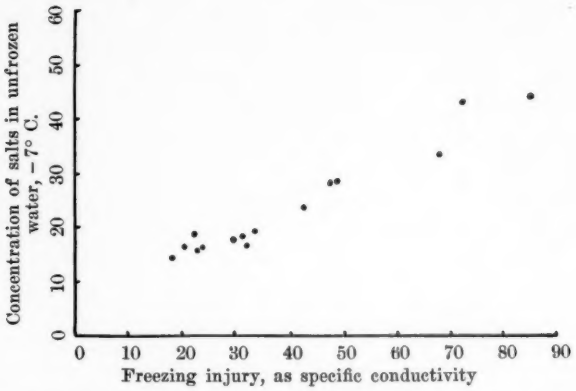

FIG. 5. Relationship between the concentration of salt in the unfrozen water in the plant and the injury due to freezing (as measured by exosmosis of salts, following freezing) is shown in the diagram. The points plotted are those given in table IV, for the −7° C. series. High correlation between the two values is indicated by the almost linear form of the scatter-diagram.

relatively high in organic material as hardening proceeds in the field seems unquestionable from these data. The same decrease in soluble electrolytes was found, however, in plants grown in the greenhouse in sand cultures where a low concentration of mineral nutrients could not occur in the soil. Furthermore this decrease appeared to occur whether nitrogen was present in the cultures or not, and, in minus-nitrogen plants, took place in the dark, where hardening also occurred; but it did not take place in plus-nitrogen plants in the dark, where hardening failed to take place. Further studies are being made with greenhouse material.

In order to study further the relation of total minerals to hardiness as measured by the freezing-exosmosis method, measurements were made on 35 varieties of hybrid wheats which were seeded in six replicate rows on the University farm by the Agronomy Department. In the 35 varieties were included a few well-known varieties as indicators. Samples of crowns were used, as before, and weighed 5 grams. Percentage of dry matter was determined as previously stated. The samples were frozen in test-tubes; and exosmosis of mineral matter following freezing was measured in the usual way. The samples were taken, killed by heating, and extraction went on for 24 hours before measurements of total minerals were made. Determinations were made on three samples from each variety, all being obtained when the plants were in a fairly well hardened condition. The values for total extractable minerals and for freezing-exosmosis were averaged for the three samples. Figure 6 shows a scatter diagram of the relationship

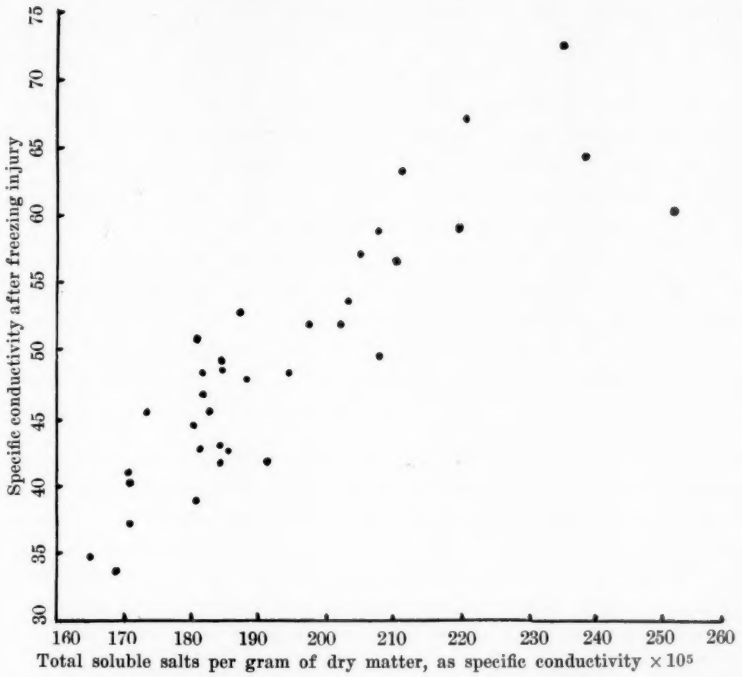

FIG. 6. Five-gram samples (triplicates) of crowns of 35 varieties of winter wheat were used. Exosmosis of salts into 25 cc. of water after freezing was measured conductimetrically to give the values on the vertical axis. The samples were then killed by heat. Diffusion of salts into the water continued for 24 hours when conductivity measurements were taken. These values, divided by the dry weight of the sample are shown on the horizontal axis.

between total extractable salts per gram of dry matter and conductivity resulting from exosmosis after freezing. The varieties of known hardiness fall, in this diagram, approximately where they would be expected, that is, hardiness was associated with low exosmosis of minerals following freezing, and with small amounts of soluble salt per gram of dry matter.

The figure indicates a considerable correlation between the values used. If extractable salts were plotted against percentage of dry matter, instead of against freezing-injury, a more or less similar diagram would result. That is, low percentage of dry matter is associated with high soluble mineral content and with low hardiness.

The total extractable salts in the samples of wheat crowns varied only perhaps 20 per cent., however, from the tenderest to the hardiest used in

this experiment. The extractable salts per gram of dry matter varied about 40 per cent. On the contrary, the salts extractable following freezing may vary several hundred per cent., depending upon the severity of freezing treatment used. Following very severe freezing treatment, extraction of salts is nearly the same as extraction following killing with heat, since differential freezing-injury is obscured by the heavy injury to hardy and tender varieties alike.

Discussion and summary

1. The data presented here show that withdrawal of water by ice-formation was not a fully reversible process in the wheat crowns used in this study. However, as the plants became more hardy, the process became more nearly reversible. This is in accord with observation of plants which have been frozen, either with or without injury. If they have been frozen beyond recovery, the water removed from the cells by ice-formation is not reabsorbed to give the previously turgid tissue.

2. Less water was left unfrozen in the tenderer varieties, but perhaps the difference is no more than would be expected on an osmotic basis from the slight differences in the freezing-point depression of the saps. The water left unfrozen at $-7°$ C. is, however, much greater than can possibly be explained on an osmotic basis from computations from the freezing-point depression of the boiled, expressed juice (LEBEDINCEV, **12**). At $-13°$ C. the excess unfrozen water is considerably smaller. That is, freezing at the lower temperature appeared to "unbind" the "bound" water (BRIGGS, **2, 3**).

3. As the season advanced and the plants became more hardy, the soluble mineral content of the tissues decreased in all four varieties. Since the dry matter in the samples increased, the soluble salt content per gram of dry matter decreased several fold. The concentration of soluble salts in the unfrozen water decreased very markedly, since the amount of unfrozen water tends to increase as hardiness increases. This was true with each variety as it hardened. It was also true that there was a higher salt concentration in the unfrozen water of the tender varieties than of the hardy ones.

4. In 35 winter wheat varieties studied, there was found a considerable inverse correlation between hardiness and soluble salts. In general, hardiness was associated with a high percentage of dry matter, and with low concentration of soluble salts.

Further studies are being carried on with other species of plants to determine whether these trends in mineral concentration are usually associated with the hardening process. An attempt is being made to determine what becomes of the soluble salts as hardening proceeds.

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EFFECT OF SOIL TEMPERATURE ON TRANSPIRATION IN *HELIANTHUS ANNUUS*

F. E. Clements and E. V. Martin

(with four figures)

Introduction

In the researches upon the adaptation of transplants to alpine and dune habitats, it has been essential to develop field methods for analyzing the factor complex. Chief among these are control batteries of phytometers in which one major factor is maintained in a series of intensities or levels while the others are held practically uniform, except for the daily march. Water content, light, wind, and soil temperature in particular permit such manipulation, and all are under investigation in the series of transplant gardens at Pike's Peak and at Santa Barbara. In both the alpine climax and the xerosere of litoral dunes, instrumentation has shown that wind and soil temperature are relatively extreme, and much attention has in consequence been directed to them.

The first experiments in this field were conducted by Whitfield (**12, 13**) at the Alpine Laboratory in 1930–1931 by means of standardized phytometers of *Helianthus annuus,* and the native *Mertensia sibirica,* which is much utilized in the transplant project. He demonstrated that a reduction of soil temperature from 113° to 51° F. lowered transpiration very little, while a decrease from 51° to 34° F. exerted a pronounced influence. Records of soil temperature during the growing season at 12,000 feet in the alpine tundra of Pike's Peak indicated a range from 35° to 48° F., with an average of 44° F. for the four years of observation (**14**).

A physiological test indoors of the relation between temperature and absorption was long ago made by Sachs (**7**), who placed a well-watered tobacco plant in a warm room and surrounded the pot with ice. After a brief period the plant began to wilt, but when the ice was removed and the soil heated, it recovered without the addition of water. By means of potometers, Vesque (**10**) found that within the limits of 10° to 15° C. the absorption by roots increased rapidly as the temperature rises. Kozarow (**3**) employed the same method to show that a fall from 20° to 0° C. retarded the rate of water supply by 25–30 per cent. Later investigations by Delf (**2**), Stiles and Jørgensen (**9**), and by Weber and Hohenegger (**11**) agree in demonstrating that the rate of absorption by a plant cell always increases with a rise of temperature, at least up to 30° C. Such responses in absorption are naturally reflected in transpiration, although Stahl (**8**) has discovered that temperatures near 0° C. may still permit a slight loss of water.

Methods

For the earlier series, plants of *Helianthus annuus* were grown from seed in water-tight, cylindric, galvanized iron cans 8″ in diameter and 10″ in height, while for later ones similar cans $8\frac{1}{2}'' \times 11''$ were employed. Each of these was fitted with a removable lid that had in its center a circular opening 2″ in diameter. The soil used was a good loam of as nearly uniform texture and moisture as was feasible. The holard was set at approximately 65 per cent. of saturation (or 28 per cent. of the dry weight of the soil), and the total amount of water in each can was kept within 15 per cent. of this value throughout the growing period. When necessary, water was added through a glass tube extending into a layer of gravel about 1″ thick in the bottom of the can. When the plants were about six weeks old, the containers were sealed by adding a layer of sand about $\frac{1}{2}''$ thick on top of the soil, filling the opening in the lid with non-absorbent cotton, and corking the glass tube. The efficiency of this type of seal was tested by means of controls without plants. Weighings made on a torsion balance to the nearest gram during the time the experiments were in progress showed that such containers seldom lost a detectable amount. This seal has the advantage over the paraffin type in that forced aeration is unnecessary (CLEMENTS and GOLDSMITH, 1).

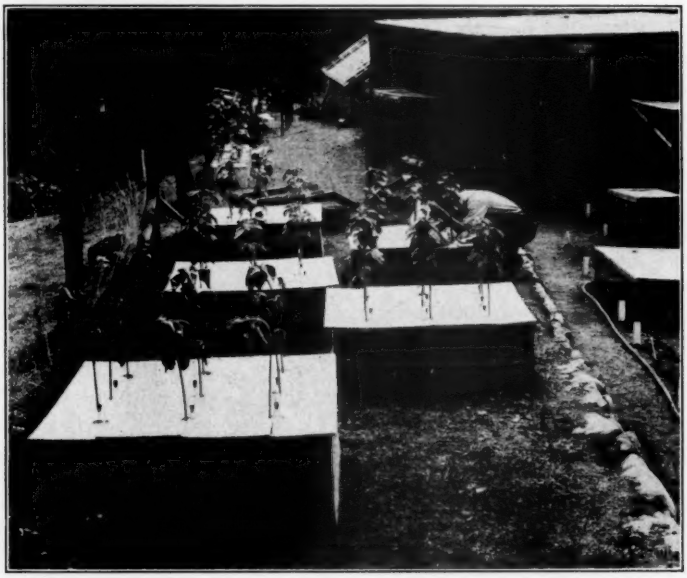

FIG. 1. Experimental installations as used in the later series, showing wilted condition of plants in a soil temperature of 30° F.

TABLE I

SOIL TEMPERATURE AND TRANSPIRATION

SERIES (1933)	AVERAGE SOIL TEMP.	INDIVIDUALS PER SET	TIME	TRANSPIRATION OF SET	SET LEAF AREA	TRANSPIRATION
	° *F.*		*hrs.*	*gm.*	*dm.²*	*gm./dm.²/hr.*
April 27	99	4	4.06	330	52.6	1.55
	73	.....	.....	295	53.1	1.37
	55	.....	.....	271	49.8	1.34
	46	.....	.....	254	54.5	1.15
	41	.....	.....	223	55.4	0.99
May 3	97	4	4.00	369	60.2	1.53
	74	.....	.....	339	51.5	1.65
	55	.....	.....	367	57.0	1.61
	47	.....	.....	318	53.9	1.47
	37	.....	.....	156	57.1	0.68
May 4	100	4	4.04	564	51.5	2.72
	75	.....	.....	485	60.2	1.99
	56	.....	.....	433	53.9	1.99
	48	.....	.....	348	57.1	1.51
	36	.....	.....	109	57.0	0.47
Aug. 25	99.5	9	4.31	379	60.3	1.46
	75	.....	.....	385	54.5	1.64
	55.5	.....	.....	374	58.0	1.50
	46	.....	.....	311	55.7	1.30
	36	.....	.....	143	58.8	0.56
Aug. 31	55.5	9	3.78	389	65.2	1.58
	51	.....	.....	382	62.9	1.61
	46	.....	.....	359	65.0	1.46
	40	.....	.....	272	69.0	1.04
	34	.....	.....	52	65.2	0.21
Sept. 2	52.5	9	4.09	835	68.3	2.98
	47	.....	.....	870	70.2	3.03
	42	.....	.....	752	72.3	2.54
	37.5	.....	.....	399	68.8	1.42
	34	.....	.....	138	68.8	0.49
Sept. 4	53	9	4.13	893	73.2	2.95
	47.5	.....	.....	910	72.5	3.04
	43	.....	.....	825	72.4	2.76
	38	.....	.....	562	69.6	1.81
	34.5	.....	.....	265	72.1	0.71

In the earlier series, five sets of four plants each were used, and these were placed in water baths 20″ square and 9″ deep, while in the later ones, five sets of nine plants each were employed with baths 3′ square and 10″ deep (fig. 1). In all cases these baths were held at fixed temperatures by additions of ice or hot water as occasion demanded, and any influence of the water on the immediate environment of the shoots was prevented by placing on the boxes closely fitting lids constructed of masonite or celotex.

On the day before a series was run, the phytometers were weighed and brought to their proper water content. At 6:00 A.M. the next day they were placed in the water baths, allowed four hours in which to become adjusted, and were then weighed to the nearest gram at 10:00 A.M. and again at 2:00 P.M. Soil temperatures were maintained constant throughout the series, and were registered by thermometers inserted near the centers of the cans. After the 2:00 P.M. weighing, the plants were taken out of the baths to permit recovery from any effects of the soil conditions, so as to be ready for future series. Leaf areas were determined in square decimeters by multiply-

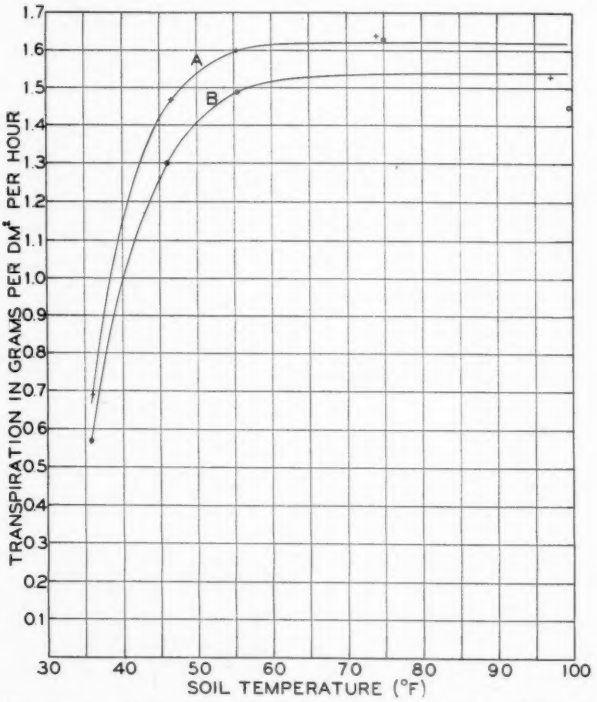

FIG. 2. Graphical representation of results from series of May 3 (A) and August 25 (B).

ing the product of length and width of the leaves in centimeters by the factor 0.013, according to the method of CLEMENTS and GOLDSMITH (1).

Results

In table I are given the data taken on seven different days, and in figure 2 the results for May 3 and August 25, 1933, are represented graphically

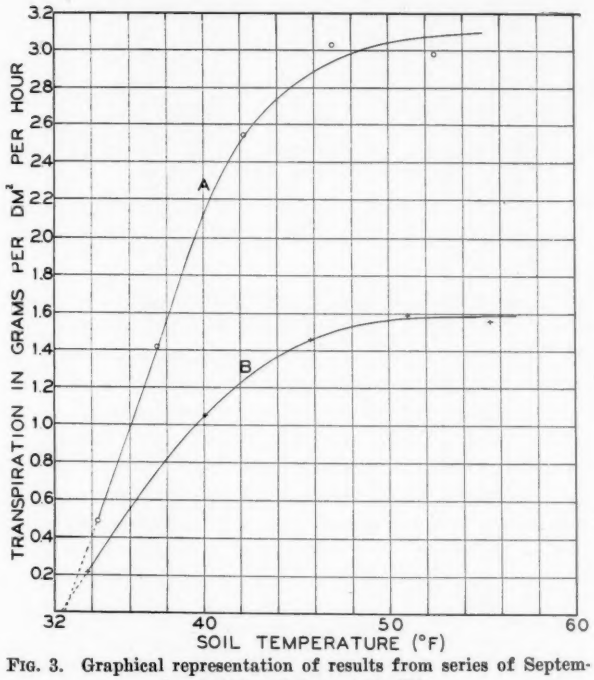

FIG. 3. Graphical representation of results from series of September 2 (A) and August 31 (B).

by curves A and B respectively, which portray transpiration in grams per square decimeter per hour (ordinate) as a function of soil temperature in degrees Fahrenheit (abscissa). Similarly, the data for September 2 and August 31, 1933, are represented in figure 3 by curves A and B respectively. The differences between the results for different days are due partly to the fact that the plants vary in age and partly to diversity in environmental factors (table II). Air temperature and relative humidity were measured at regular intervals during the various series, while the average wind velocity was obtained from the readings of a Weather Bureau four-cup anemometer. The light intensity was obtained from an estima-

TABLE II

METEOROLOGICAL DATA

DATE	AVERAGE AIR TEMPERATURE	AVERAGE RELATIVE HUMIDITY	AVERAGE WIND VELOCITY	LIGHT, ESTIMATED % FULL SUNLIGHT
	° *F.*	%	*m.p.h.*	%
April 27	65	49	1.9	25
May 3	73	24	3.5	100
May 4	80	27	4.1	100
Aug. 25	65	53	2.4	75
Aug. 31	66	45	2.0	50
Sept. 2	74	12	2.5	100
Sept. 4	75	21	2.1	100

tion of the relative lengths of time of cloudiness and full sunlight during the experiments.

The degree of opening of the stomata was secured by taking strips of lower epidermis of the leaves, fixing them in absolute alcohol, and observing them under a microscope (**6**). Since the stomatal aperture varied considerably within a narrow region of epidermis, only three degrees of opening are considered, namely, open, partially closed, and either narrow slits or closed; the results of these observations are given in table III. Since the condition as to wilting was closely connected with the degree of opening of the stomata, this also was noted and included in the same table. Under ordinary conditions, wilting begins with a soil temperature of about 40° and increases as the latter decreases to 32° F., while the stomata begin to close at about 40° and are nearly completely closed at 37°.

Accuracy of results

Errors in this type of experiment arise mainly from two sources, the experimental method and the differences between individual plants. The latter are caused principally by variations in growth or structure and differences in shading of lower leaves by the upper ones, while the former includes errors in the phytometer method, in leaf measurements, and in the weighing itself, as well as those arising from the length of time required to weigh the entire group of plants, which in these series amounted to about 25 minutes.

In order to discover the approximate values of the errors due to these various causes, 52 phytometers of *Helianthus annuus* were grown under similar conditions, both of soil and atmosphere, up to seven weeks of age. These were sealed as described and weighed at 10:00 A.M. and 2:00 P.M. on each of three successive days. The water content was maintained ap-

TABLE III

STOMATAL OPENING AND WILTING

DATE	TIME	AVERAGE SOIL TEMPERATURE					REMARKS ON WILTING
		99°	73°	55°	46°	41°	
April 27	10:00	Open	Open	Open	Open	P. Cl.*	Leaves of plants at 41° were a little less turgid than normally
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		97°	74°	55°	47°	37°	
May 3	10:00	Open	Open	Open	Open	Sl.–Cl.	Plants at 37° somewhat wilted; edges of leaves curled a little
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		100°	75°	56°	48°	36°	
May 4	10:00	Open	Open	Open	Open	Sl.–Cl.	Plants at 36° were wilted so that the crowns bent over a little
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		99.5°	75°	55.5°	46°	36°	
Aug. 25	10:00	Open	Open	Open	Open	Sl.–Cl.	Plants at 36° were wilted so that the crowns bent over a little
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		55.5°	51°	46°	40°	34°	
Aug. 31	8:00	Open	Open	Open	Open	P. Cl.	Leaves of plants at 40° a little less turgid than normal; plants at 34° wilted so that crowns bent over considerably
	9:00	"	"	"	"	"	
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	
		52.5°	47°	42°	37.5°	34°	
Sept. 2		Strips unsatisfactory					Leaves of plants at 37.5° drooped and curled a little; plants at 34° wilted so that the crowns bent at nearly right angles to the main stem (fig. 1)

TABLE III—(*Continued*)

STOMATAL OPENING AND WILTING

DATE	TIME	AVERAGE SOIL TEMPERATURE					REMARKS ON WILTING
		53°	47.5°	43°	38°	34.5°	
Sept. 4	8:00	Open	Open	Open	Open	Open	Leaves of plants at 38° drooped and curled a little; plants at 34.5° wilted considerably, but crowns did not bend over
	9:00	"	"	"	"	P. Cl.	
	10:00	"	"	"	P. Cl.	Sl.–Cl.	
	11:00	"	"	"	"	"	
	12:00	"	"	"	"	"	
	1:00	"	"	"	"	"	
	2:00	"	"	"	"	"	

* P. Cl., partially closed; Sl.–Cl., openings are either slits or are closed. Measurements of the stomatal openings for the earlier series were made by DR. F. L. LONG of the Carnegie Institution of Washington.

proximately constant by adding the proper amount each day. Leaf areas were measured on the first and third days, and the averages of these two were taken as the values for the intermediate day.

The average transpiration in grams per square decimeter per hour for the 4-hour period and the deviations of the individuals from this value were calculated. Since it required 30 minutes to weigh the 52 phytometers, the transpiration of the first plant weighed was measured for the period 10:00–2:00, while that of the last plant was from 10:30–2:30, the others falling between these two extremes. Since no drift in the deviations of the individuals was discernible, it was concluded that any error caused by lack of simultaneity of weighing was within the limits imposed by other sources. An approximate value of the error introduced by the latter was obtained in the following manner from the deviations of the individuals. The 52 phytometers were divided at random into 13 sets of four each, and the average transpiration for each set was determined for each of the three days. The maximum deviation of any one set from the average of the 13 sets for each day was 9 per cent., while the average deviation was 3 per cent. Similarly, when the 52 plants were grouped into sets of nine each, the maximum deviation observed was 4 per cent. and the average 1.5 per cent. It is probable, however, that the data obtained with the soil temperature experiments are not altogether as accurate as these figures indicate, since a slight error may arise from the effect of the water baths on the air about the plants, especially when the lids are lifted. In the measurement of temperature there may be appreciable errors due partially to gradients within the baths and partly to fluctuations about the average. Such deviations can only be estimated, but it is probable that the averages given in table I are in error by not more than about 1° F.

Discussion

In representing the data by smoothed curves as in figures 2 and 3, it is assumed that the relation between soil temperature and transpiration is a continuous one, and this assumption seems to be justified by the accuracy with which the curves fit the points. As can be seen from the curves, transpiration varies very little with soil temperatures between 55° and 100° F., but falls off below about 55° and is reduced to half at about 38°. Extrapolation of the curves in figure 3 indicates that transpiration approaches zero at 32.5°, although the accuracy of the data is not sufficient to fix this point at 32.5° and not 32° as is to be expected.

Obviously the explanation of this reduction in transpiration with soil temperatures below 55° F. is that the absorbing capacity of the root system is lowered, thus reducing the water supplied to the leaves. This usually does not produce a noticeable loss of turgor until the temperature has been decreased to about 40° F. The degree of wilting increases as the temperature is reduced below 40°, and at 34° has proceeded so far that the crowns of the plants are bent at nearly right angles to the main stems (table III). This wilting did not continue long enough to cause the plants any permanent injury, as was shown by the following test: On August 25, 1933, the set of plants at 36° F. (table I) was removed from its bath at 2:50 P.M. and placed in the 75° bath. This set and the one at 55.5° were weighed again at 4:00 P.M. and at 5:00 P.M. During the period from 2:50 to 4:00 the losses were 1.02 and 1.55 gm./dm.^{2}/hr. respectively, while during that from 4:00 to 5:00 the losses were 0.48 and 0.51 gm./dm.^{2}/hr. respectively. For this latter period the set that had wilted lost practically as much water as the set that had not. Also, by 4:00 P.M. the soil temperatures of both sets were about 55°, and none of the plants gave any visual signs of having been wilted.

The condition of the stomata during the various series is indicated in table III. Only three degrees of opening are considered, open, partially closed, and either narrow slits or closed, denoted in the table by the symbols Open, P.Cl., and Sl.–Cl., respectively. The stomata did not start to close until the soil temperature had been lowered to the point where the plants began to wilt perceptibly, about 40° F., but they were almost completely closed at 37°. These results indicate that it is not necessary for the stomata to close in order for transpiration to be reduced. For example, at 45° F. the transpiration was lowered appreciably, but the stomata showed no signs of closing; hence, the reduced water loss in this case must be due to a reduction in the water supplied to the leaves, which is in turn caused by a decrease in the absorbing capacity of the root system. Whether the amount of water transpired represents the amount absorbed during the same period of time is questionable, but since the degree of wilting of the

plants appeared to remain constant throughout the weighing period, it seems probable that such is the case.

KRAMER (**4**) points out that absorption of water by roots may be affected by temperature in two ways: first, by the physical effects (largely increased viscosity and decreased vapor pressure) which result in a slower movement of water from soil to root; and second, by the physiological effects on the permeability of the root cells. He gives data showing that the absorption of water from loam by soil-points (LIVINGSTON and KOKETSU, **5**) at 60° F. is approximately twice that at 32° F. Whether these results can be considered as a measure of the physical effects of low soil temperature on absorption of water by roots is questionable; but if so, then the results obtained with phytometers of *Helianthus annuus* indicate that the physiological effects are of greater importance than the physical.

The data presented here agree with those of the earlier workers already mentioned, and they also constitute an addition to present knowledge in that the effect upon transpiration of soil temperature has been traced through the critical region 34° to 55° F. So far as is known, this range has not heretofore been investigated.

The applicability of these results to alpine problems is definitely limited in that the plants were grown under soil temperatures of 60°–70° F., and the effect of lower soil temperatures upon transpiration has been measured for only the first few hours after the plants were subjected to those conditions. However, since WHITFIELD (**12**) has shown that transpiration of phytometers of *Helianthus annuus* in alpine regions (12,000 feet on Pike's Peak) may be as much as 2 gm./dm².hr., the results of curve B of figure 3 are applicable to this climatic region. Soil temperatures in this same climate are represented graphically in figure 4 (taken from WHITFIELD, **14**),

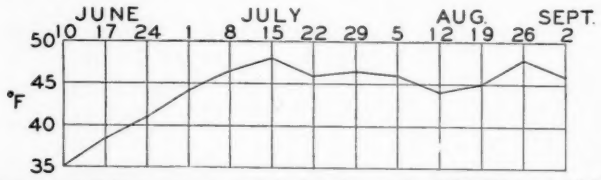

FIG. 4. Average soil temperature at 4 inches, by weeks 1927–1930, at 12,000 feet on Pike's Peak (from WHITFIELD, 1933).

which shows that there is a variation between 35° and 48° F. for the growing season. Accordingly our results indicate that, in this particular alpine climate at least, soil temperature probably has an appreciable influence on transpiration and growth of vegetation. These results are obtained with phytometers of *Helianthus annuus*, however, and it is possible that native alpine plants would respond differently.

The final test of this influence remains to be carried out. Experiments are planned in which plants will be grown in the field from seeds or seedlings under various soil temperatures with other environmental factors similar, and the growth, transpiration, and other functional activities will be measured. This problem will require several thermostatically controlled baths, but it is expected to yield considerable information concerning the effect of low soil temperature on vegetation.

Summary

1. The effect of soil temperatures in the range 34°–100° F. on transpiration of *Helianthus annuus* phytometers 6–8 weeks old was measured by using five sets of four or nine plants each, run simultaneously in water baths of different temperatures.

2. It was found that transpiration varies very little with soil temperatures between 55° and 100°, but drops rapidly below 55°, was reduced to half at 38°, and approached zero at 32°.

3. The temperature of the baths was held constant within 1° F., and some data are presented that indicate an accuracy in transpiration measurements of from 3 to 5 per cent.

4. Strips of lower epidermis of the leaves show that the stomata do not begin to close until the soil temperature is lowered to about 40°, but are nearly completely closed at 37° F.

5. The plants usually start to wilt at about 40° and at 34° are wilted so that the crowns are bent at nearly right angles to the main stems.

6. The plants recover rapidly from the wilted condition when the soil temperature is raised.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

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RELATION OF BENZOIC ACID CONTENT AND OTHER CONSTITUENTS OF CRANBERRIES TO KEEPING QUALITY[1]

J. A. Clague and C. R. Fellers

Introduction

The benzoic acid naturally occurring in cranberries has long been regarded as the preserving agent responsible for the good keeping qualities of the berries and the products manufactured from them (**10, 15**). The purpose of this investigation was to determine the benzoic acid content of several Massachusetts, New Jersey, and Wisconsin varieties, and to note the amount present in relation to other constituents and to the keeping qualities of the berries.

Loew (**8**) was probably the first to report the presence of benzoic acid in the German Preisselbeere (*Vaccinium vitis idaea*). Subsequent quantitative determinations were reported by other workers (**5, 7, 9**). Griebel (**4**) thoroughly studied the free and combined benzoic acid of the Preisselbeere, the Moosbeere (*V. oxycoccus*), and the American cranberry (*V. macrocarpum*). He found the Preisselbeere to contain as much as 0.22 per cent. total benzoic acid, the Moosbeere and the cranberry having a content of 0.02–0.06 per cent. Mason (**10**) found the American cranberry to contain 0.05 per cent. benzoic acid; Radin (**16**) reports 0.06 per cent. as the content; Blatherwick and Long (**2**) give the percentage as 0.096; and Nelson (**14**) reports 0.069 per cent. The varieties analyzed were not stated in the papers. Chemical composition of the important American varieties of cranberries has been studied by Morse (**12**).

Experimental methods

The cranberries were shipped to the laboratory in quarter-barrel boxes and were put into cold storage (2° C.) upon receipt, samples being taken out as needed for analysis.

The procedure followed in the determination of benzoic acid was for the most part that recommended by the A. O. A. C. (**1**). A modification was made in the treatment of the chloroform extract. The residue from the latter was dissolved in ether and transferred to test tubes and treated in the manner described by Monier-Williams (**11**). The ether was evaporated under a stream of air dried by bubbling through concentrated sulphuric acid. The residue was washed down from the sides of the tubes until it was contained in the bottom 2 or 3 cc. Some previously ignited sand was added and a mark filed on the test tubes about 4 cm. from the

[1] Contribution No. 195 of the Massachusetts Agricultural Experiment Station.

bottom. A disk of filter paper was pushed down to the mark. A 1-gallon slip cover can was used as a sublimator. The test tubes were pushed through holes in an asbestos mat which fitted over the can, the test tube base resting on a wire basket inside the can, so that the filed mark was just below the surface of the asbestos mat. Cork stoppers were placed loosely over the mouth of the test tubes and they were heated at 160°–170° C. for about two hours. After cooling, the bottom 4 cm. of the tubes were cut off at the mark. The crystals of benzoic acid were all sublimated in the upper part of the tube. Any that had fallen down were caught on the filter paper disc, which was removed and from which the crystals were shaken back into the upper part of the test tube. The test tubes with the sublimate were dried in a desiccator over sulphuric acid and then weighed. The benzoic acid was washed into a small Erlenmeyer flask with alcohol, and the test tubes again weighed. The difference in weight represents the benzoic acid present in the sample. The sublimate washed out of the test tube was titrated with 0.05 N sodium hydroxide, thus giving a check on the gravimetric determination. This modified method gave very reliable results and reduced the time required to make a determination.

Total acids and pectin, as alcohol precipitate, were determined by the A. O. A. C. (**1**) methods.

The soluble solids content of the juice from new crushed cranberries was determined with the Abbé refractometer. Results are not so accurate as those obtained by gravimetric methods, but give good comparative data.

The number of berries per quart container was considered to be the most convenient measure for their size. The weight of a quart of berries represents the average weight of ten quarts.

Results

The benzoic acid content of 24 varieties is shown in table I. Results for two years are given for four varieties. The percentage of the berries spoiled at the time of analysis is shown. The rating of varietal keeping qualities as given by C. S. Beckwith of the New Jersey Cranberry Station, H. J. Franklin of the Massachusetts Station, and C. M. Chaney of the American Cranberry Exchange is also shown.

With one exception (Berry Berry variety) the percentage of benzoic acid was over 0.05, the maximum being 0.098.

According to Cruess and Irish (**3**), fruit products with a pH of 3 to 3.5 require less than 0.05 per cent. sodium benzoate (equivalent to about 0.04 per cent. benzoic acid) for preservation. In grape products, where the pH was 2.6 and 3.0, the equivalent of 0.02 per cent. benzoic acid sufficed to preserve the fruit against yeast spoilage.

The pH of sound cranberries is 2.35–2.6 (**17**); hence preservative con-

TABLE I

VARIETAL DIFFERENCES IN CHEMICAL COMPOSITION AND KEEPING QUALITY

VARIETY	SOURCE	BERRIES PER QUART	WEIGHT PER QUART	ESTIMATED KEEPING QUALITY	DECAYED AFTER 4 MONTHS AT 35° F.	BENZOIC ACID		TOTAL ACID	PECTIN AS ALCOHOL PRECIPITATE	SOLUBLE SOLIDS BY REFRACTOMETER
						1931–32	1932–33			
			oz.		%	%	%	%	%	%
Berry Berry	Massachusetts	237	13.2	Poor	43	0.029		2.08	1.06	8.2
Bugle		343	12.8	Good–excellent	18	0.068		2.77	1.29	8.6
Centennial		239	12.6	Poor–fair	80	0.062		2.55	1.18	9.2
Early Black		408	12.9	Fair–very good	80 (soft)	0.055	0.071	2.51	0.91	7.2
Holliston		238	11.8	Poor	30	0.054		2.29	1.4	8.0
Howes		358	13.7	Very good	15 (soft)	0.072	0.08	2.27	1.24	9.2
Maxim Randall		320	12.7	Good–very good	16	0.063	0.084	2.46	1.14	8.2
Maxim Randall (Admixture)		297	12.8	Good			0.067	2.46	0.90	9.2
Shaw's Success		396	14.0	Good–very good	6	0.077	0.098	2.55	1.17	9.2
Shurtliff		295	12.2	Fair	50	0.077		2.38	1.22	8.8
Smalley Howes		423	13.0	Good	6	0.076		2.80	1.13	8.6
Tom Howes		324	12.8	Fair			0.072	2.27	0.86	9.6
Braddock Bell	New Jersey	322	12.7	Good	24	0.080		2.25	1.03	9.8
Budd's Blues		600	13.4	Excellent	10	0.066		2.17	1.23	10.6
Early Richards		360	12.8	Poor–fair	35	0.091		2.56	1.08	9.2
Harold		325	12.5	Excellent	25	0.053				
Howard Bell		316	12.8	Fair	25	0.079		2.22	1.15	9.6
Nancy Munyon		508	12.7	Fair	50	0.074		2.20	1.10	8.8
Plum		450	14.3	Good–very good	31	0.083		2.31	1.66	8.6
Woolman's		327	12.3	Good	17	0.073		2.19	0.95	9.8
Bennett's Jumbo	Wisconsin	333	12.7	Good–very good	12	0.068		2.68	1.36	9.6
Berlin		463	13.6	Good–very good	37	0.077		2.43	1.30	9.2
Gebhardt's Beauty		356	13.0	Good	50	0.088		2.46	1.41	9.8
Searls Jumbo		395	13.1	Poor–fair	40	0.077		2.28	1.04	9.8
Average		360	12.9		32	0.065	0.079	2.35	1.16	9.1

ditions in cranberries are apparently ideal. However, annual loss from fruit rot does constitute as much as 25 per cent. of the total cranberry crop of the United States (**18**).

There seems to be no definite relationship between the keeping quality of cranberries and their benzoic acid content. Factors other than the latter must be considered. The infection by spoilage microorganisms usually occurs relatively early in the growing season (**19**). The benzoic acid is present only in traces in the immature berries and the amount increases gradually to a maximum as the berry ripens (**4, 10**). Benzoic acid exerts a preservative rather than a germicidal action on microorganisms, in the concentrations in which it is present in the ripe cranberry. If the infective agent has already established itself, therefore, the gradually increasing quantities of the acid apparently are not sufficient to destroy the fungi.

Furthermore, GRIEBEL (**4**) has pointed out that some of the benzoic acid in cranberries is combined as esters and as a glucoside of vacciniin. As much as 35 per cent. of the total benzoic acid was in the form of the glucoside in some American cranberries analyzed by GRIEBEL. It is not known whether this combined form of benzoic acid exerts any antiseptic action.

The percentage of total acids (calculated as citric) in the cranberries analyzed varied from 2.08–2.80, with an average of 2.35. Of the nine varieties listed as only fair or as poor keepers, six had total acid contents below the average and one was only 0.03 per cent. above. The keeping qualities of 15 varieties were rated from good to excellent. Nine of these had total acid contents above and six below the average.

Pectin as alcohol precipitate was found to vary between 0.86 and 1.66 per cent., averaging 1.16 per cent. No definite relationship existed between keeping quality and pectin content. The average soluble solids content was 9.1 per cent., with a maximum of 10.6 and a minimum of 7.2.

Some of the best keeping varieties had a high soluble solids content, the variety Budd's Blues having the maximum amount and having a rating as excellent. On the other hand, some of the berries which were poor keepers also had a high percentage of soluble solids.

EFFECT OF QUINIC ACID AS A PRESERVING AGENT

Cranberries are known to have a quinic acid content of as much as 1 per cent. (**6, 13**). The following test was made to determine the efficiency of quinic acid as an antiseptic, and also to note the effect of the combination of quinic and benzoic acids.

To one series of tubes containing sterile sweet cider there was added 2 per cent. quinic acid plus 0.1, 0.05, and 0.025 per cent., and no sodium benzoate. A similar series was made with the addition of 1 per cent. quinic acid and a third contained the sodium benzoate alone. The media were

inoculated with yeasts and molds and incubated at room temperature. Results are shown on table II.

TABLE II

PRESERVATIVE EFFECT OF QUINIC AND BENZOIC ACIDS

Quinic acid per cent.	2	2	2	2	1	1	1	1	0	0	0
Benzoic acid per cent.	0.1	0.05	0.025	0	0.1	0.05	0.025	0	0.1	0.05	0.025
Yeasts	−	−	−	+	−	−	−	+	−	−	+
Molds	−	−	−	+	−	−	−	+	−	−	−

After three days' incubation there was abundant growth in all tubes containing the quinic acid alone, and no growth in the others. At the end of ten days the only change was that yeast was growing in the tube containing 0.025 per cent. sodium benzoate alone. The increased acidity of the media containing the added quinic acid probably accounted for this difference. No further changes were noted after a month's incubation period. It was concluded that the naturally occurring quinic acid did not exert an appreciable antiseptic or germicidal action on fruit spoilage organisms.

Summary and conclusion

1. The benzoic acid content of twenty-four varieties of cranberries ranged from 0.029 to 0.098 per cent., with an average of 0.065 per cent.

2. The varieties with the best keeping qualities did not always have a high benzoic acid content, so that apparently factors other than benzoic acid content alone must be largely responsible for the keeping properties of the berries.

3. The quinic acid present did not exert any appreciable preservative action on spoilage organisms.

4. The large percentage of varieties having poor keeping qualities had low total acid content, while the good keeping varieties in the majority of cases had a high total acid value.

5. Pectin and soluble solids content did not correlate with keeping quality.

6. Benzoic acid is present in the ripe berry in amounts sufficient to give a preservative action. However, high benzoic acid content in a variety is not always indicative of good keeping quality; apparently certain physical or environmental factors are of greater importance.

MASSACHUSETTS STATE COLLEGE
AMHERST, MASSACHUSETTS

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EFFECT OF ETHYLENE, ETHYLENE CHLOROHYDRIN, AND ULTRA-VIOLET LIGHT ON CARBOHYDRATE CONTENT OF STORED APPLES[1]

ROBERT B. DUSTMAN

Literature review

In 1924 CHACE and DENNY (**2**) described the use of ethylene as a new and satisfactory method for the coloring of citrus fruit. Since that time various investigators have reported the effect of this and other substances upon the general ripening processes of fruits and vegetables. Differences of opinion have arisen concerning the effect of these ripening agents upon the color changes and the chemical composition of the treated fruits and vegetables. REGEIMBAL and HARVEY (**9**) found a decrease in total sugars and an increase in direct-reducing sugars in pineapples treated with ethylene or propylene. They suggested that the increase of direct-reducing sugars arises from sucrose and concluded that the activity of the proteolytic enzymes and invertase of pineapples is increased by treatment with ethylene or propylene. ENGLIS and ZANNIS (**3**), however, reported no acceleration of taka-diastase action on soluble starch when the solutions were treated with ethylene. This was true also for the diastase of corn meal in acting upon the starch of the grain. The rate of hydrolysis of sucrose by invertase likewise showed no increase for ethylene treatment.

In the case of celery blanched with ethylene, HARVEY (**4**) states that "by taste and by chemical analysis treated celery was found to be higher in sugar content than untreated celery. It was found, also, that tomatoes treated with ethylene were sweeter than untreated fruits." With tomatoes he reported a frequent initial appearance of the red color at the stem end with subsequent spreading from this region in ethylene-treated fruits. KOHMAN (**6**), however, could not confirm this color behavior but reported that, while color development might be hastened with ethylene, it proceeded in the normal manner from the blossom end. Also, HIBBARD (**5**) found that although ethylene destroys chlorophyll and thus blanches celery, chemical studies show that ethylene-treated celery is not sweeter than celery blanched by boards, and the hearts and stalks of ethylene-treated celery contain less total sugars than check lots. With tomatoes, chemical analyses showed a much higher total sugar content for vine-ripened fruits than for those artificially ripened, but a similar comparison between ethylene-ripened and air-ripened tomatoes gave no significant difference. This is said to be at

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 126.

variance with ROSA's (**10**) findings that ethylene-treated tomatoes are sweeter than those air-ripened. Later ROSA (**11**) reported no increase in sugar content of Honey Dew and Casaba melons picked slightly unripe and exposed to ethylene treatment, but the treated melons did show a marked acceleration in the rate of softening, in change of green to yellow color of the rind and in conversion of reducing sugars to sucrose.

CHACE and CHURCH (**1**) obtained no appreciable alteration of the composition of the edible portion of citrus fruits treated with ethylene, but did find an acceleration in the color development. The same treatment hastened the coloring of persimmons, destroying the astringency and producing softening.

RAMSAY and MUSSO (**8**) state that in experiments with oranges, total sugar content was not increased by treating the fruit with ethylene but actually was slightly diminished. They report also "an increase in fruit sugar, however, from the hydrolization of the cane sugar present—a process the reverse of ripening."

WOLFE (**12**) studied the effect of ethylene on the ripening of bananas and found only a very slight increase in sugars and decrease in starch, from day to day, resulting from the ethylene treatment. The treated fruit turned yellow at a somewhat more rapid rate than did the controls.

HARVEY (**4**) states that "when green apples are intended for cider or vinegar it will probably be advantageous to sweeten them by the use of ethylene. It should be remembered that starches are rather quickly digested under the ethylene treatment, so that better quality in the cider may be produced by the increase in sugars as well as by the removal of the excess fruit acids." No data on composition are given.

HIBBARD (**5**) reports an experiment in which ethylene was used on McIntosh apples in order to study its effect on color. The apples were large and of fancy grade except for lack of uniform color. They were given daily treatments of ethylene over a 2-weeks' period. The treatment had no effect on the development of red color but did destroy the green in the green colored areas, leaving them yellow. No analyses are reported.

The investigation about to be described was carried out for the purpose of obtaining data on the effect of ethylene on the composition of stored apples, special attention being given to the carbohydrate group. Treatment with ethylene chlorohydrin and irradiation with ultraviolet rays were also included for purposes of comparison.

Materials and methods

In October, two bushels of each variety of apple were picked from trees of Stayman Winesap, Rome Beauty, and Ben Davis. Each variety was picked from a single tree only and removed to cold storage (33° to 36° F.)

within 4 hours after picking. The fruits when taken from the trees were selected for uniformity of size, firmness, color, and maturity. On March 6, four lots were chosen from each variety, each lot consisting of five apples. Again at this time every effort was made to select uniform sound fruits, the general condition of all three varieties being excellent.

Investigation

TREATMENT

The various lot treatments for each variety were given daily (except Sundays) as follows:

Ethylene, 1 to 1000 by volume; ethylene chlorohydrin, 1 cc. of 40 per cent. solution per bottle (allowed to evaporate); irradiation with a universal model Hanovia mercury arc lamp for 60 minutes (30 minutes on each side) at 30-cm. distance. The lots were brought from cold storage each day except Sundays, the containers thoroughly irrigated with fresh air, the treatments repeated, and the fruits returned to storage within 1½ to 2 hours. Control lots were carried to and from storage, irrigated, removed from their containers, exposed to room temperatures during the same periods, replaced, and otherwise handled as nearly like the treated lots as could be done.

The containers used were 7-liter, wide-mouthed glass bottles equipped with rubber stoppers. The actual number of daily treatments given was 35, extending over a period of 41 days. At the end of this period the cores of all fruits were removed with a cork borer, and diagonal quarters taken from each of the five apples in each lot for chemical analysis. At the same time the half-apple remnants were used for the immediate determination of pH of the expressed juices. This was accomplished by using a Leeds and Northrup Type K potentiometer with a quinhydrone electrode, after squeezing out the juice rapidly through muslin cloth in a small hand-press.

CHEMICAL ANALYSES[2]

The samples taken for analysis were preserved in the usual manner. They were weighed, sliced rapidly, and stored after heating in approximately 80 per cent. alcohol in glass bottles. On removal from storage they were evaporated to dryness in open dishes and dried in a vacuum oven for 48 hours at 65° C. and at a pressure 27 to 27½ inches of mercury below atmospheric. After drying, the samples were ground in an agate mortar by hand.

Extraction was carried out first with ether and then with 80 per cent. alcohol, using Soxhlet extractors with glass thimbles and ground glass joints. The total extraction period for each solvent was 24 hours.

[2] Acknowledgment is gratefully made to Mr. L. C. SHRIVER, former Assistant in Agricultural Chemistry, for much of the chemical work herein reported.

The alcoholic extracts were evaporated in the usual manner and the sugar solutions cleared with neutral lead acetate and potassium oxalate.

Reducing sugars were determined directly on an aliquot of the cleared solution, using Soxhlet's modification of Fehling's solution, and the results calculated as invert sugar.

Total sugars were similarly determined after hydrolysis with HCl at 37.5° C. (A.O.A.C. method, 7). The difference between the total and the reducing sugars was multiplied by the factor 0.95 and designated as sucrose.

Starch was determined by gelatinizing the residues from the alcoholic extractions with hot water, digestion with taka-diastase at 37.5° C. for 24 hours, filtration, and subsequent hydrolysis with HCl. The reducing values were calculated as glucose. This value multiplied by the factor 0.90 was designated as starch.

Hemicellulose was determined on the residues from the starch determination. These were treated with 1 per cent. HCl on the boiling water bath for three hours. During this treatment the flasks were equipped with reflux condensers and completely immersed. Following hydrolysis, the filtrates were cooled, neutralized, transferred, made up to volume, and the reducing power calculated as glucose.

Results

The effect of the treatments described on the chemical composition of the fruits is shown in table I and II.

TABLE I

FRACTIONAL COMPOSITION AND pH VALUES OF APPLES VARIOUSLY TREATED

VARIETY	TREATMENT	TOTAL MOISTURE	pH OF EXPRESSED JUICE	MOISTURE-FREE BASIS	
				ETHER EXTRACT	SOLUBLE IN 80 PER CENT. ALCOHOL
		%	*pH*	%	%
Ben Davis	None	84.9	3.59	5.93	72.40
	Ethylene	84.6	3.53	6.17	72.67
	Ethylene chlorohydrin	84.2	3.67	5.78	73.72
	Ultraviolet	85.2	3.57	5.05	73.69
Stayman Winesap	None	81.7	3.38	5.26	79.65
	Ethylene	81.3	3.32	5.45	79.65
	Ethylene chlorohydrin	82.0	3.44	3.58	81.04
	Ultraviolet	82.1	3.43	3.90	81.04
Rome Beauty	None	86.4	3.43	5.29	77.31
	Ethylene	86.6	3.60	3.34	79.42
	Ethylene chlorohydrin	87.5	3.55	3.84	78.97
	Ultraviolet	86.2	3.58	3.78	78.81

TABLE II

CARBOHYDRATE CONTENT OF APPLES VARIOUSLY TREATED; MOISTURE-FREE BASIS

VARIETY	TREATMENT	REDUCING SUGARS AS INVERT SUGAR	TOTAL SUGARS AS INVERT SUGAR	SUCROSE (0.95 × DIFFERENCE)	STARCH (0.90 × GLUCOSE VALUE)	HEMICELLULOSE AS GLUCOSE
		%	%	%	%	%
Ben Davis	None	47.94	61.85	13.21	0.48	2.89
	Ethylene	49.72	62.58	12.22	0.51	2.22
	Ethylene chlorohydrin	50.80	63.01	11.60	0.59	2.20
	Ultraviolet	49.85	63.92	13.37	0.51	2.12
Stayman Winesap	None	57.31	71.07	13.07	0.31	1.07
	Ethylene	57.26	72.16	14.16	0.52	0.80
	Ethylene chlorohydrin	58.88	70.09	10.65	0.81	1.25
	Ultraviolet	57.64	71.48	13.15	0.61	1.41
Rome Beauty	None	57.10	71.15	13.25	0.43	0.85
	Ethylene	57.64	71.55	13.21	0.55	1.17
	Ethylene chlorohydrin	57.77	72.64	14.13	0.62	0.99
	Ultraviolet	58.02	73.39	14.60	0.53	0.91

After about four weeks, the ethylene-treated fruits were beginning to show a more rapid change of ground color from green to yellow than the untreated fruits. By the close of the experiment this color change was quite noticeable, and in addition a considerable mellowing and softening effect was apparent when the fruits were sampled for chemical analysis.

By the last days of March some of the Ben Davis apples treated with ethylene chlorohydrin were showing slight signs of scald, but this did not become pronounced during the remaining days of the experiment. The Stayman and Rome varieties were not so affected, but all three varieties under ethylene-chlorohydrin treatment showed some softening as compared with the controls.

The only noticeable effect of the ultra-violet irradiation was a very slight bronzing of the most directly exposed portions of the fruits, where the color was green.

At the close of the experiment the untreated apples of all three varieties were firm, sound, and practically unchanged in ground color.

Discussion

Examination of the values in both tables reveals a remarkably close agreement in the results from the variously treated and untreated lots. This is true even for such complex groups as the alcohol-soluble and hemicellulose fractions. The values for water content are likewise uniform for each variety. Collectively considered this uniformity of composition in treated and untreated fruits indicates the uniform character of the original material

used. The period of treatment was purposely prolonged and the storage temperature held low, with the hope that any changes occurring as a result of the ethylene or other treatments might be cumulative, and hence less transitory and more easily detected and measured.

The values for the various carbohydrate groups in table II lend scant support to the hypothesis that ethylene treatment increases the sugar content of stored apples. The variations are no greater than might be expected from uniformly treated lots.

With ethylene treatment, the sucrose of Stayman Winesap was slightly increased but it was reduced in like amount in Ben Davis and essentially unchanged in Rome Beauty. Conversely, reducing sugars were slightly increased in Ben Davis but practically unchanged in Stayman Winesap and Rome Beauty.

The ethylene-chlorohydrin treatment appears to have reduced moderately the sucrose content in Stayman Winesap and Ben Davis, and slightly increased their reducing sugars, but the change is not pronounced and does not occur at all in Rome Beauty.

The starch content is low in all three varieties but is no less in the ethylene and ethylene-chlorohydrin treated fruits. Clearly neither ethylene nor ethylene chlorohydrin has reduced the starch content, the effect, if any, being in the opposite direction.

The acidity of the expressed juice indicates that again there is nothing to show that ethylene has affected the pH value of the apples. This measure also runs uniform. Since the apples were well matured when picked, this feature might not be expected to change materially, except perhaps to increase in pH value somewhat as softening proceeded.

The outcome of these experiments has resulted in failure to increase either the sugar content or the pH value of the juices of apples held in storage. Ethylene treatment did hasten the change of color from green to yellow, however, and also the rate of softening of the apple tissues. Other experiments with ethylene treatment at higher temperatures, not otherwise described in this report, gave similar results with respect to color change and softening but in a much shorter period of time.

Summary

Ethylene, ethylene chlorohydrin, and ultraviolet irradiation treatments of stored apples of Rome Beauty, Stayman Winesap, and Ben Davis varieties did not materially affect the chemical composition of the fruit or the pH value of the expressed juices as compared with similarly stored, untreated fruits. Ethylene treatment hastened the color change from green to yellow and likewise accelerated the softening of the apple tissues.

DEPARTMENT OF AGRICULTURAL CHEMISTRY
WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION
MORGANTOWN, W. VA.

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LONG AND SHORT WAVE-LENGTH LIMITS OF PHOTOSYNTHESIS[1]

G. RICHARD BURNS

(WITH TWO FIGURES)

A complete description of the apparatus and the experimental procedure employed in these experiments has been given elsewhere (**1**). Briefly, it consisted of exposing the plant to one portion of the spectrum for two hours, determining the amount of carbon dioxide used, and then exposing it to such an intensity of a second portion of the spectrum that the amount of carbon dioxide used was the same as in the first case. Under this condition it was assumed that the same amount of photosynthesis had occurred in the two cases, and that the relative efficiency of the two radiations in causing photosynthesis was inversely proportional to their intensities. In the experiments described in this paper, the method employed to determine the limits of photosynthesis was to compare the amount of photosynthesis caused by radiation—well within the limits—with that caused by radiation about half of which was within the limit and half just outside. For example, the short wave limit was obtained by comparing the efficiency of radiation 5 (fig. 1) with radiation 4, and the long wave limit by comparing 5 with 2. The different portions of the spectrum were obtained by providing each of the light sources with a double filter cell, one compartment being 1 inch thick and the other 0.5 inch thick. The solution in the 1-inch cell was circulated and cooled at the rate of 5 cubic feet per hour. Table I gives the filters used, table II and figure 1 the transmission spectra of these filters. The wave-lengths given are the wave-lengths at the center of the thermopile at each measurement, the spectrum having been shifted the width of the thermopile between each measurement. Thus in table II the

TABLE I

FILTERS

COMPOSITION OF SOLUTIONS

No.	1-INCH CELL	0.5 INCH CELL
1	Water	Water
2	Water	50 gm. $K_2Cr_2O_7$ in 1 liter water
3	62 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water	Water
4	62 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water	500 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water
5	62 gm. $CuSO_4 \cdot 5\ H_2O$ in 1 liter water	50 gm. $K_2Cr_2O_7$ in 1 liter water

[1] Contribution from the Vermont Agricultural Experiment Station, published with the consent of the Director.

eighth line, beginning 6461, means that between about 6696 and 6272 Å., filter 1 transmitts 105.3 parts of energy; filter 2, 102.0 parts; filter 3, 1.9 parts; etc.

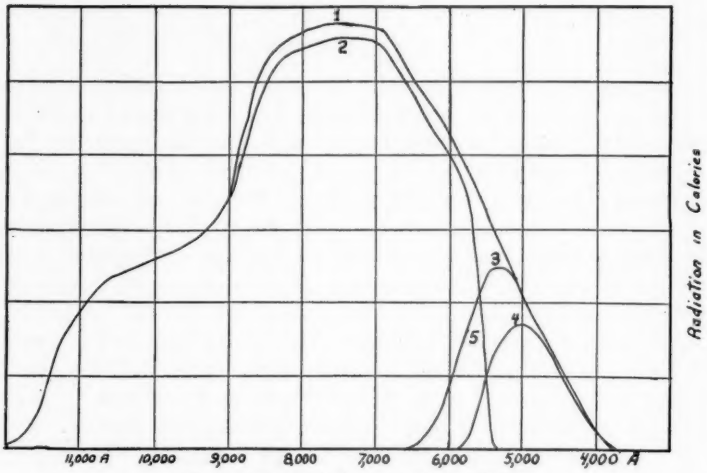

FIG. 1. Transmission spectra of filters.

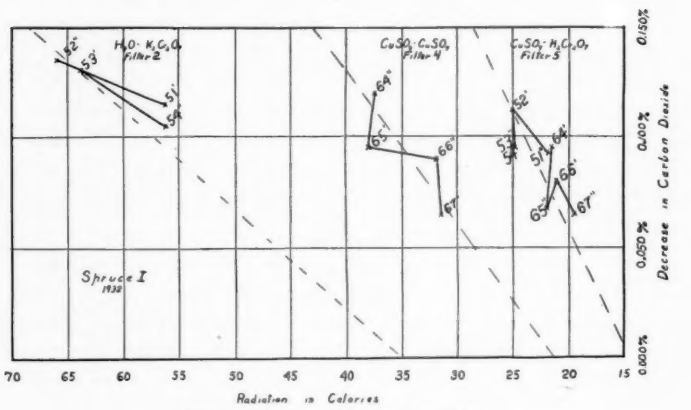

FIG. 2. Graph of typical results.

In table III the run number is the serial number of the day on which the experiment was performed (July 1 being 1) and the number of primes indicates whether it was the first or second determination on the tree that day. The second column gives the number of the tree; P1 for white pine number

TABLE II

TRANSMISSION OF FILTERS

WAVE LENGTH	FILTER 1	FILTER 2	FILTER 3	FILTER 4	FILTER 5
Å	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
14270	0.4	0.2	.....	.....	.....
12230	6.4	6.4	.....	.....	.....
10610	116.0	116.0	.....	.....	.....
9380	162.5	162.0	.....	.....	.....
8280	249.0	242.0	.....	.....	.....
7519	194.0	188.0	.....	.....	.....
6931	150.0	146.0	.....	.....	.....
6461	105.3	102.0	1.9	.....	1.3
6082	76.3	73.5	12.5	0.2	12.1
5769	56.0	50.4	25.4	3.0	24.2
5502	40.9	16.6	28.2	10.7	12.9
5270	29.7	0.5	24.4	16.2	0.2
5071	21.6	.....	19.6	16.2	.....
4898	15.8	.....	14.8	13.6	.....
4743	11.8	.....	11.3	10.7	.....
4602	9.0	.....	8.4	8.1	.....
4480	6.6	.....	5.6	5.8	.....
4368	4.7	.....	4.0	4.2	.....
4267	3.4	.....	2.9	2.9	.....
4173	2.5	.....	2.0	2.0	.....
4088	1.6	.....	1.3	1.4	.....
4010	1.1	.....	1.1	1.1	.....
3936	0.7	.....	0.4	0.4	.....
3867	0.4	.....	0.2	0.2	.....
3803	0.1	.....	0.1	0.1	.....

1, S1 for Norway spruce number 1, etc. The next column gives the filter used; the next two the decrease in carbon dioxide concentration expressed as percentage total gas (duplicate determinations). The column headed radiation is the radiation in calories per cm.^{2} per run $\times$ 10. These values are not corrected for the transmission of the bell-jars, about 89 per cent. The corrected radiation is the radiation that would be necessary to obtain exactly the same carbon dioxide decrease as in the companion run, and was determined from a graph of the values (fig. 2). The next column gives the percentage efficiency of one of the portions of the spectrum as compared with the other, and the final column shows the experimental error which would result from an error of 0.005 per cent. in the gas analysis and the maximum probable error in the graphical determination of the corrected radiation value.

In considering table III, it is interesting to note the change in efficiency exhibited by a tree toward a given portion of the spectrum on successive days. While the change is usually less than 10 per cent., it may be as high as 50 per cent. for the first two or three days of exposure to a new radiation:

TABLE III

Run	Tree	Filter	Carbon dioxide decrease (duplicate determinations)		Radiation	Corrected radiation	Efficiency	Experimental error
					Cal. × 10	*Cal.* × 10	%	%
7′	P1	5	0.120	0.120	32.9	.....	.....	.....
7″	P1	3	0.135	0.135	32.5	31.2	106.0	3
8′	P1	3	0.180	0.175	40.6	43.3	86	4
8″	P1	5	0.200	0.190	37.4	.....	.....	.....
9′	P1	5	0.285	0.290	46.2	.....	.....	.....
9″	P1	3	0.235	0.240	49.2	56.4	82	4
11′	P1	5	0.345	0.340	50.5	.....	.....	.....
11″	P1	3	0.290	0.280	53.0	60.5	83	4
12′	P1	3	0.260	0.250	48.4	50.0	75	2
12″	P1	5	0.270	0.260	37.7	.....	.....	.....
14′	P1	5	0.365	0.365	50.9	.....	.....	.....
14″	P1	3	0.250	0.255	51.7	69.5	73	5
7′	P2	5	0.045	0.045	31.8	.....	.....	.....
7″	P2	3	0.050	0.050	29.3	28.4	112	3
8′	P2	3	0.090	0.085	36.0	53.5	79	8
8″	P2	5	0.185	0.185	42.5	.....	.....	.....
9′	P2	5	0.200	0.210	47.3	.....	.....	.....
9″	P2	3	0.185	0.190	50.9	54.3	87	3
11′	P2	5	0.280	0.270	56.2	.....	.....	.....
11″	P2	3	0.240	0.245	55.5	60.5	93	3
12′	P2	3	0.180	0.180	44.1	49.0	90	4
12″	P2	5	0.210	0.215	42.4	.....	.....	.....
14′	P2	5	0.290	0.290	49.8	.....	.....	.....
14″	P2	3	0.215	0.220	53.1	64.2	78	5
16′	P3	5	0.215	0.215	39.4	.....	.....	.....
16″	P3	3	0.240	0.240	44.8	41.4	95	4
18′	P3	3	0.145	0.145	30.0	27.0	79	7
18″	P3	5	0.120	0.125	21.3	.....	.....	.....
19′	P3	5	0.240	0.245	31.9	.....	.....	.....
19″	P3	3	0.245	0.245	38.0	37.7	85	2
20′	P3	3	0.170	0.170	33.3	31.5	82	4
20″	P3	5	0.155	0.155	25.8	.....	.....	.....
21′	P3	5	0.200	0.200	29.7	.....	.....	.....
21″	P3	3	0.205	0.210	32.2	31.3	93	5
22′	P3	3	0.170	0.170	30.5	34.8	75	6
22″	P3	5	0.210	0.210	26.0	.....	.....	.....
16′	P4	5	0.195	0.200	37.7	.....	.....	.....
16″	P4	3	0.205	0.200	34.5	34.2	110	10
18′	P4	3	0.135	0.140	28.4	25.0	90	5
18″	P4	5	0.105	0.110	22.4	.....	.....	.....
19′	P4	5	0.230	0.225	31.4	.....	.....	.....
19″	P4	3	0.205	0.210	34.6	36.7	86	3
20′	P4	3	0.190	0.190	32.4	31.5	93	3
20″	P4	5	0.180	0.180	29.4	.....	.....	.....
21′	P4	5	0.245	0.250	34.2	.....	.....	.....
21″	P4	3	0.190	0.190	34.2	40.8	84	4
22′	P4	3	0.165	0.165	30.4	33.7	81	5
22″	P4	5	0.200	0.195	27.4	.....	.....	.....
23′	P1	3	0.160	0.160	38.1	33.8	.....	.....
23″	P1	4	0.135	0.135	40.3	.....	84	6
27′	P1	4	0.190	0.195	45.2	.....	85	8
27″	P1	3	0.280	0.280	49.1	38.4	.....	.....
29′	P1	3	0.175	0.175	33.5	33.3	.....	.....

TABLE III—(*Continued*)

Run	Tree	Filter	Carbon dioxide decrease (duplicate determinations)		Radiations	Corrected radiation	Efficiency	Experimental error
					Cal. × *10*	*Cal.* × *10*	%	%
29″	P1	4	0.170	0.175	39.9	.....	83	3
30′	P1	4	0.305	0.305	69.2	.....	79	2
30″	P1	3	0.295	0.295	53.2	54.6	.....	.....
31′	P1	4	0.105	0.115	33.7	.....	85	3
31″	P1	3	0.120	0.115	29.7	28.8	.....	.....
32′	P1	3	0.210	0.210	43.3	40.0	.....	.....
32″	P1	4	0.185	0.185	50.3	.....	80	3
23′	P2	3	0.125	0.125	40.1	40.5	.....	.....
23″	P2	4	0.125	0.130	44.3	.....	91	2
27′	P2	4	0.195	0.195	49.2	.....	88	7
27″	P2	3	0.225	0.230	49.5	43.6	.....	.....
29′	P2	3	0.175	0.170	34.8	30.3	.....	.....
29″	P2	4	0.140	0.135	41.8	.....	72	6
30′	P2	4	0.280	0.275	70.6	.....	85	4
30″	P2	3	0.250	0.250	55.8	60.0	.....	.....
31′	P2	4	0.105	0.105	36.7	.....	80	6
31″	P2	3	0.125	0.120	31.9	29.5	.....	.....
32′	P2	3	0.135	0.135	42.1	51.3	.....	.....
32″	P2	4	0.190	0.185	51.8	.....	99	8
38′	P3	3	0.140	0.140	16.9	18.7	.....	.....
38″	P3	1	0.165	0.155	65.2	.....	28.7	8
39′	P3	1	0.295	0.295	107.2	.....	44.4	14
39″	P3	3	0.145	0.150	29.8	47.6	.....	.....
40′	P3	3	0.210	0.210	41.8	47.0	.....	.....
40″	P3	1	0.250	0.245	91.3	.....	51.5	4
41′	P3	1	0.170	0.175	72.7	.....	45.9	2
41″	P3	3	0.175	0.175	33.6	33.3	.....	.....
42′	P3	3	0.180	0.180	35.3	34.4	.....	.....
42″	P3	1	0.170	0.175	65.7	.....	52.3	2
43′	P3	1	0.250	0.250	84.9	.....	55.0	4
43″	P3	3	0.220	0.220	42.4	46.6	.....	.....
44′	P3	3	0.225	0.220	41.8	36.8	.....	.....
44″	P3	1	0.185	0.185	75.2	.....	49.0	2
38′	P4	3	0.130	0.125	16.0	17.3	.....	.....
38″	P4	1	0.140	0.145	64.6	.....	26.8	6
39′	P4	1	0.280	0.280	109.5	.....	49.6	10
39″	P4	3	0.140	0.140	33.3	54.3	.....	.....
40′	P4	3	0.235	0.230	42.9	45.5	.....	.....
40″	P4	1	0.250	0.250	92.8	.....	49.0	3
41′	P4	1	0.170	0.170	73.6	.....	44.0	3
41″	P4	3	0.175	0.175	33.1	32.4	.....	.....
42′	P4	3	0.175	0.175	33.3	28.5	.....	.....
42″	P4	1	0.140	0.135	61.0	.....	46.7	5
43′	P4	1	0.215	0.215	85.3	.....	50.5	2
43″	P4	3	0.210	0.205	42.0	43.0	.....	.....
44′	P4	3	0.200	0.205	42.8	39.0	.....	.....
44″	P4	1	0.175	0.175	76.9	.....	50.7	5
49′	S1	2	.....	.....	.....	.....	.....	.....
49″	S1	5	.....	.....	.....	.....	.....	.....
50′	S1	5	0.005	0.005	12.3	21.7	.....	.....
50″	S1	2	0.110	0.115	53.6	.....	40.5	16
51′	S1	2	0.115	0.115	57.2	.....	41.6	5
51″	S1	5	0.095	0.090	21.9	23.8	.....	.....
52′	S1	5	0.115	0.115	24.9	26.8	.....	.....

TABLE III—(*Continued*)

Run	Tree	Filter	Carbon dioxide decrease (duplicate determinations)		Radiations	Corrected radiation	Efficiency	Experimental error
					Cal. × *10*	*Cal.* × *10*	%	%
52″	S1	2	0.135	0.135	66.0	.....	40.6	4
53′	S1	2	0.130	0.130	63.9	.....	45.1	6
53″	S1	5	0.095	0.095	24.8	28.8	.....	.....
54′	S1	5	0.095	0.095	24.8	26.0	.....	.....
54″	S1	2	0.105	0.105	56.2	.....	46.3	4
49′	S2	2	.....	.....	.....	.....	.....	.....
49″	S2	5	.....	.....	.....	.....	.....	.....
50′	S2	5	0.050	0.050	11.6	17.7	.....	.....
50″	S2	2	0.160	0.155	54.6	.....	32.7	25
51′	S2	2	0.060	0.060	58.7	.....	36.0	3
51″	S2	5	0.050	0.050	20.1	21.1	.....	.....
52′	S2	5	0.060	0.060	24.0	27.0	.....	.....
52″	S2	2	0.090	0.090	62.7	.....	43.0	4
53′	S2	2	0.110	0.110	62.5	.....	41.6	4
53″	S2	5	0.085	0.085	23.9	26.0	.....	.....
54′	S2	5	0.100	0.100	24.3	24.0	.....	.....
54″	S2	2	0.095	0.100	56.2	.....	41.7	2
55′	P3	2	0.075	0.075	54.6	.....	41.7	5
55″	P3	5	0.070	0.070	22.1	22.8	.....	.....
56′	P3	5	0.085	0.085	22.3	18.6	.....	.....
56″	P3	2	0.055	0.055	44.9	.....	41.4	10
57′	P3	2	0.110	0.110	57.1	.....	39.0	4
57″	P3	5	0.105	0.105	21.8	22.3	.....	.....
58′	P3	5	0.120	0.120	21.7	21.7	.....	.....
58″	P3	2	0.120	0.120	57.7	.....	37.6	3
60′	P3	2	0.160	0.160	55.0	.....	39.1	3
60″	P3	5	0.160	0.160	21.5	21.5	.....	.....
55′	P4	2	0.110	0.115	56.0	.....	47.7	6
55″	P4	5	0.080	0.085	23.3	26.7	.....	.....
56′	P4	5	0.105	0.105	23.5	18.3	.....	.....
56″	P4	2	0.055	0.055	45.5	.....	40.2	11
57′	P4	2	0.110	0.105	61.7	.....	38.9	3
57″	P4	5	0.100	0.105	23.5	24.0	.....	.....
58′	P4	5	0.130	0.130	23.0	22.6	.....	.....
58″	P4	2	0.125	0.125	61.3	.....	36.9	3
60′	P4	2	0.175	0.175	60.6	.....	44.0	5
60″	P4	5	0.140	0.140	23.4	26.7	.....	.....
64′	S1	5	0.095	.....	22.6	24.6	.....	.....
64″	S1	4	0.120	.....	37.4	.....	65.8	4
65′	S1	4	0.095	0.095	38.0	.....	64.5	4
65″	S1	5	0.070	0.065	22.0	24.5	.....	.....
66′	S1	5	0.080	0.080	21.2	21.9	.....	.....
66″	S1	4	0.090	0.090	31.9	.....	68.7	3
67′	S1	4	0.065	0.065	31.4	.....	62.5	2
67″	S1	5	0.065	0.065	19.6	19.6	.....	.....
64′	S2	5	0.105	.....	23.4	22.6	.....	.....
64″	S2	4	0.095	.....	38.0	.....	59.5	3
65′	S2	4	0.125	0.125	39.2	.....	70.5	8
65″	S2	5	0.070	0.075	22.4	27.6	.....	.....
66′	S2	5	0.070	0.070	21.9	22.6	.....	.....
66″	S2	4	0.080	0.075	33.6	.....	67.3	3
67′	S2	4	0.035	0.035	32.4	.....	63.6	4
67″	S2	5	0.025	0.025	20.6	21.6	.....	.....

Run 39, P3, filter 3, −41%; filter 1, − 6%
Run 39, P4, filter 3, −50%; filter 1, −11%
Run 51, S1, filter 2, − 6%; filter 5, −13%
Run 51, S2, filter 2, −34%; filter 5, −42%

From the values in table III the weighted averages of table IV were obtained. The limits of photosynthesis were calculated by means of the transmission spectra of the filters and the weighted averages on the following assumptions:

1. The absorption and reflection of the plant does not change with changing wave-length. Since absorption is less and reflection greater in the center of the visible spectrum, this assumption would make the calculated limits somewhat too far apart.

2. The amount of photosynthesis is proportional to the number of quanta and not to the energy of the radiation. If the amount of photosynthesis were proportional to the energy, the red limit would be moved out about 160 Å. and the blue in about 120 Å.

TABLE IV

EFFICIENCY OF FILTERS AND LIMITS OF PHOTOSYNTHESIS

TREE	FILTERS		EFFICIENCY A IN TERMS OF B	LIMIT OF PHOTOSYNTHESIS
	A	B		
			%	Å
S1	2	5	42.8	7430
S2	2	5	40.5	7380
P3	2	5	39.7	7340
P4	2	5	41.5	7390
S1	4	5	65.4	4660
S2	4	5	65.3	4660
P1	4	5	66.1*	4650
P2	4	5	73.3*	4490
P1	3	5	80.0	4630
P2	3	5	85.4	4430
P3	3	5	82.8	4530
P4	3	5	86.0	4390
P1	4	3	82.6	4460
P2	4	3	85.8	4370
P3	1	3	49.8	7160‡
P4	1	3	48.3	7140‡
P3	1	5	41.2†	7220‡
P4	1	5	41.5†	7230‡

* Calculated from 3–5 and 4–3.
† Calculated from 1–3 and 3–5.
‡ Violet limit set at 4540.

3. The quantum yield does not change with changing wave-length.
4. The limit of photosynthesis is sharp.

These assumptions are being made the subject of further study, as is the ability of the plant to adapt itself to the use of different wave-lengths. In calculating the limits, the transmission spectra were put on an approximate quantum basis by multiplying the energy at a particular spectrograph setting by the wave-length at the center of the thermopile. The radiation intensities were multiplied by the average quanta per calorie value of the particular filter as determined from the transmission spectra.

The 2–5 and the 4–5 filter combinations (table IV) should give the most accurate results and the other values are included for comparison.

It should be noticed that P1 had the lowest value in the 3–5 runs and doubtlessly the limit obtained from P2 is more typical. An error of 10 per cent. in the efficiency would shift the values 100 Å in the red and 140 Å in the blue. On the other hand the spectrograph readings, the calculations and the filters employed should yield more accurate results in the blue. In considering the blue limit it should be remembered that WARBURG and NEGELEIN (**2**), with algae, found only a 25 per cent. decrease in the quantum yield at 4360 Å. This difference in the short wave limit is probably due to the characteristics of the plants or to the fact that the trees were purposely not acclimated to blue and violet light.

Summary

A study of the limits of photosynthesis of white pine and Norway spruce showed that these trees were able to use all of the visible spectrum with the exception of part of the blue and all of the violet.

VERMONT AGRICULTURAL EXPERIMENT STATION
BURLINGTON, VERMONT

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EVAPORATION IN ITS THERMODYNAMIC RELATION TO THE COHESION THEORY AND TO IMBIBITIONAL AND OSMOTIC PRESSURES OF CELL CONSTITUENTS

CLYDE HOMAN, T. F. YOUNG, AND CHARLES A. SHULL

(WITH THREE FIGURES)

That the upward flow of dilute solutions in the tracheae of the taller plants results from a tension in the column of liquid sustained by its cohesive forces (correlated with evaporation from the cells at the top of the column) has been postulated in the well known cohesion theory of DIXON and JOLY (**3**). The purposes of this paper are to show that the maximum tension or negative pressure in the tracheae depends on the relative humidity of the atmosphere, to show how evaporation operates to produce this tension, and to call attention to a thermodynamic equation by which possible and theoretical values may be calculated. The use of a similar equation in determining osmotic and imbibitional pressures of cell constituents and soils is also illustrated.

A simple mechanical apparatus for the production and demonstration of negative pressures produced by evaporation is shown in figure 1. The

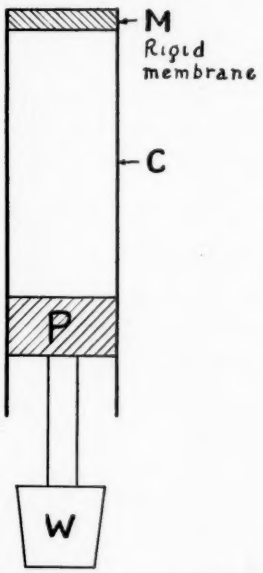

FIG. 1. Apparatus for production and demonstration of negative pressures by evaporation.

membrane, M, which is sealed to the top of the cylinder, C, contains pores which permit water to pass from the cylinder and evaporate into the atmosphere. The cylinder is filled with gas-free water. The piston, P, is free to move, but is so made that no air may leak past it. We shall neglect its own mass and that of the liquid in the cylinder, and concentrate our attention on the variable mass, W.

When water evaporates from the upper surface of the membrane, the volume of the liquid in the chamber decreases; and the piston, if free, is drawn upward. Now as we observe the rise of the piston we may increase the weight, W, until the resulting downward force has produced such a negative pressure or tension in the liquid that further loss of water by evaporation (and consequent rise of the piston) is stopped. This force divided by the area of the piston (less the atmospheric pressure acting on P) is, of course, the negative pressure within the cylinder. Should W be further increased, water vapor would condense from the atmosphere, pass through the membrane into the cylinder, and the piston would descend. The column of water in the cylinder corresponds to that in the tracheae, and the membrane corresponds to the mesophyll cells. For each set of conditions (temperature, relative humidity) there will be a unique value of the negative pressure which can be calculated by an equation first used by POYNTING (5):

$$\frac{d P_l}{d P_v} = \frac{V_v}{V_l} \tag{1}$$

in which P_l is the pressure within the liquid, P_v is the (partial) pressure of vapor above the membrane, and V_l and V_v are the molal volumes (the volume of one mole of liquid or vapor) of the liquid and gaseous water respectively.

The molal volume of the vapor may be represented approximately by: $V_v = \frac{RT}{P_v}$ in which R is the gas constant, 82.06 cc. atm. mole^{-1} deg,$^{-1}$, and T is the absolute temperature. The volume of the liquid does not vary greatly for moderate changes in pressure. If we represent it simply by a constant, V_l, equation (1) may be integrated:

$$P_l - P_l' = \Delta P_l = \frac{RT}{V_l} \ln \frac{P_v}{P_v'} = 2.3026 \frac{RT}{V_l} \log \frac{P_v}{P_v'} \tag{2}$$

Here P_v is the partial pressure of water vapor above the membrane when equilibrium is established, P_v' is the vapor pressure of the water when the pressure on the liquid is one atmosphere (1.013×10^6 dyne cm.$^{-2}$). [This is nearly the vapor pressure (within 0.1 per cent.) of H_2O when the liquid water is subjected to its own vapor pressure]. P_l' is the atmospheric pres-

sure to which P_v' corresponds, *i.e.*, one atmosphere, and P_l is the variable pressure within the liquid to which P_v corresponds. When P_v/P_v' is less than one, $P_l - P_l'$ is negative. When P_v/P_v' becomes sufficiently small, P_l itself becomes negative.

The pressure difference inducing the flow of sap within the plant is the pressure on the cells of the roots minus the pressure produced in the mesophyll sap by evaporation, *i.e.*, $P_l' - P_l$. This difference (which is one atmosphere minus the pressure, P_l) we shall designate for convenience the *stromogenic pressure difference*. When the stromogenic pressure difference is greater than one atmosphere, a negative pressure or tension exists in the liquid, and in such cases the term stromogenic *tension* may be employed.

In table I, column 2, are listed *calculated values* of the maximum stromogenic tension which can be produced in pure water by evaporation into an atmosphere in which the relative humidity has the values listed in column 1. In making these calculations we have neglected the change (with pressure) of the molal volume of water.

Extrapolating the density data of BRIDGMAN (**1**), we have also obtained the values in column 3. The corrections, involving extrapolation, may not be precise but serve to illustrate the magnitude of the errors in column 2. There are two minor approximations in the calculation. We have used the perfect gas law, and Dalton's law of partial pressure. The data given are, of course, for pure water, and not for dilute solutions, such as the soil solu-

TABLE I

MAXIMUM STROMOGENIC PRESSURES AT 20° C.* FOR VARIOUS RELATIVE HUMIDITIES

RELATIVE HUMIDITY	STROMOGENIC TENSION	
	CALCULATED, NEGLECTING VARIATIONS IN V_l	CORRECTED FOR VARIATIONS IN V_l
%	*atm.*	*atm.*
99.9	1.33	1.33
99.5	6.68	6.68
99.0	13.40	13.39
98.0	26.93	26.91
95.0	68.4	68.3
90.0	140.4	140.0
70.0	475	471
50.0	924	906
30.0	1605	1550
10.0	3069	2880

* According to the equation, the usual variations in temperature would affect the pressures but little, as these are proportional to the absolute temperature.

tion and sap in the tracheae. For such dilute solutions appropriate corrections could be applied, for the vapor pressure of water from the solution, and for the difference in the molal volume (partial molal volume—see later) of water in the solution.

It is not likely that (negative) pressures within liquid water can ever reach the higher values indicated in the table. The greatest tension obtained experimentally in water by DIXON (**2**) was 158.4 atmospheres, and in plant sap 207 atmospheres. Higher values, however, may be possible, and theory indicates that they are.

Osmotic and imbibitional pressures

Figure 2 represents an idealized apparatus for illustrating two convenient methods for applying and measuring osmotic and imbibitional pres-

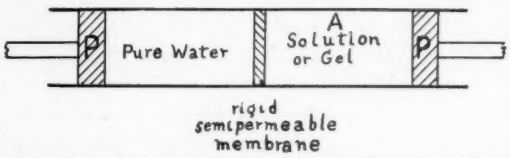

FIG. 2. Apparatus for applying and measuring osmotic and imbibitional pressures.

sures. The first, the usual method, is to maintain the pressure on the pure liquid constant, and to apply and measure the pressure which must be applied to A to prevent osmosis through the semipermeable membrane at the center. The second method is to maintain the solution or gel at a constant pressure, and decrease the pressure on the pure liquid B (to negative values if necessary) until osmosis or imbibition is prevented. The two values so obtained are not in general exactly the same, since the effect of a decrease in pressure on the molal volume is not the same as an increase, and because the molal volume of the pure liquid is not the same as the partial molal volume[1] of the solvent in the solution. The differences are small for dilute solutions, and we shall refer later to the values determined by the second method, *i.e.*, that involving a reduction of the pressure on the pure solvent.

The water in every solution or gel having a given osmotic or imbibitional pressure, Π, would be in equilibrium with an atmosphere of some definite

[1] The partial molal volume, $\overline{v}$, as defined by G. N. LEWIS (**4**) is the increase in volume per mole of water (or any other substance) added when the amount added is infinitesimal, *i.e.*, $\overline{v} = dV/dn$ where V is the volume of the solution containing n moles of water. $\overline{v}$ is not difficult to determine experimentally. Like the molal volume of the pure liquid, it is a function of pressure and temperature. It is also a function of the composition of the solution, and differs very little from the molal volume of the pure liquid when the solution is dilute.

relative humidity which may be calculated. If Π represents the osmotic pressure of the solution as measured by method 2, then the water in the solution is in equilibrium with water under the negative pressure Π. The partial pressure of water vapor in equilibrium with the latter can be calculated from equation (2). Since the solution must be in equilibrium with water vapor at the same partial pressure, it follows that the osmotic pressure Π of any solution is related approximately to the relative humidity of the atmosphere in equilibrium with it, by the equation:

$$\Pi = -\frac{RT}{V_1} \ln \frac{P_v}{P_v'} \qquad (3)$$

This is a well known thermodynamic equation.

Consider for example a solution, the osmotic pressure of which is 68.3 atmospheres. Such a solution placed in the right chamber of figure 2 would of course be in equilibrium with water in the left chamber under a tension of 68.3 atmospheres. The latter would be in equilibrium with the atmosphere when the relative humidity is 95 per cent. (see table I), and the solution is in equilibrium with the same atmosphere.

If a negative pressure is applied to a solution the osmotic pressure of which is not negligible, the relative humidity corresponding may be calculated approximately (accurately for dilute solutions at low pressures) by use of equations (2) and (3) together. Suppose the solution whose osmotic pressure is 68.3 atmospheres is placed in the chamber of figure 1. If the relative humidity is reduced from 95 to 90 per cent., to prevent evaporation the weight, W, would have to be such that a tension of approximately 140 – 68.3, or 71.7 atmospheres were produced in the cylinder. If no pressure were applied, evaporation would go on until the concentration of the solution corresponded to an osmotic pressure of 140 atmospheres. If the relative humidity were raised from 95 to 99 per cent., a pressure of $68.3 - 13.4 = 55$ atmospheres would have to be exerted by the piston P. In the absence of any such pressure, water would be absorbed from the atmosphere, and the solution would become more dilute until its osmotic pressure had been reduced to 13.4 atmosphere.

Figure 3 will be used to illustrate some of the points brought out previously in connection with mesophyll cells abutting on tracheae. A is a membrane, as in fig. 1, but it is not rigid and is free to move up and down the cylinder. B is a semipermeable membrane held rigid, while P is a piston as in fig. 1. If compartment AB contains a solution whose osmotic pressure is 68.3 atmospheres, if the relative humidity above the membrane A is 95 per cent. (see table I), and if a pull equivalent to 68.3 atmospheres is exerted by W on piston P, the system will remain stationary. Should the relative humidity drop to say 90 per cent., however, membrane A will move

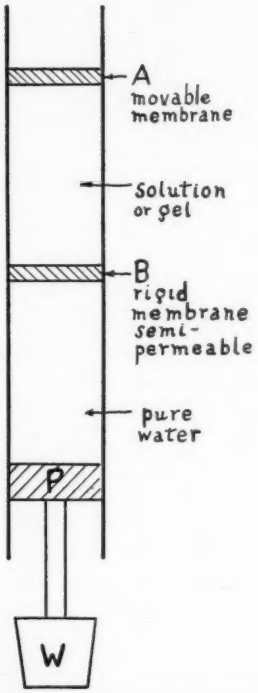

FIG. 3. Apparatus model to illustrate the action of mesophyll cells.

downward as the concentration and the osmotic pressure increase. The piston P will then move upward as water diffuses through membrane B and evaporates from membrane A. If W, however, is increased at the time the relative humidity is decreased to 90 per cent., until a pull of 140 atmospheres is exerted, water will pass downward through B and evaporation will take place from A (which would move downward) until the osmotic pressure becomes 140 atmospheres due to increasing concentration. Equilibrium would then be reached. Thus it is that were it not for delay in reaching equilibrium resulting from slowness of diffusion, the stromogenic tension and the osmotic pressure of the cell vacuoles would be constantly the same, and in equilibrium with each other.

Since diffusion through cell walls and in and out of submicroscopic pores in the walls and through stomatal pores must be a very slow process, whereas the flow of sap may be relatively rapid, it is probable that, under actual conditions, nowhere near the theoretical maximum pressures are ever produced in active tissues. In addition, external and internal conditions are

constantly changing, and calculations made from the relative humidity of the external air could not be taken as a basis for calculating actual stromogenic tensions, etc., but would indicate only the possible tensions that could be produced, were all conditions suitable. They do show, however, the high reserve in lifting ability over what even the tallest trees require. Even in low-growing plants a considerable portion of this reserve lifting ability may be utilized at times to overcome friction in the tracheae, etc., and to withdraw water from dry soils in which water is held at high imbibitional pressures.

Stromogenic tension at wilting point of leaves

Since the relative humidities of natural atmospheres are frequently as low as 20 to 30 per cent., it is evident that the construction of plants enables them to prevent the evaporation of water to a marked degree, or they would quickly perish. It is possible that extreme xerophytes (*Opuntia* spp., etc.), and possibly epiphytes may be the only types of plants that could continue to live and grow in an atmosphere containing water at very low partial pressure. Mesophytes would promptly wilt when the water supply is shut off at the roots and the internal atmosphere drops only a small amount below saturation. In such types 68 atmospheres would be a high average value for the osmotic pressure of the cell vacuoles, and wilting would occur at 20° C. when the internal atmosphere of the leaf reached a relative humidity of 95 per cent. (assuming that the osmotic pressure is 68 atmospheres). Since the relative humidity of the external atmosphere is much below 95 per cent., it is obvious that the epidermal cells with their cutinized surfaces and stomatal pores must operate efficiently in maintaining the humidity of the atmosphere surrounding the thin-walled mesophyll cells in a state of approximate saturation.

It would no doubt be possible to determine the relative humidity of the internal atmosphere of the leaf at the time of wilting, which would be closely related as described previously to the maximum possible stromogenic tension which these leaves could develop at wilting. The detached leaves could be put into a closed chamber and the point determined at which a decrease in the relative humidity produced an observable loss of turgor, while an increase in humidity restored the loss. (The definiteness of this point would vary with different leaves, and it is conceivable that certain types of leaves would not readily indicate the loss of turgor in the internal cells). The relative humidity could be found by analysis of a sample of the air from the chamber, or by calculation from the water content of sulphuric acid placed in the chamber. The use of a slightly pulsating pressure in the chamber might be desirable to hasten the approximation of equilibrium conditions.

Application to soils

Suppose a sample of soil were placed in a closed container and allowed to come to equilibrium with air of some selected relative humidity. If the relative humidity were then lowered, water would be lost by the soil. The loss, which could be determined by weight, would represent the amount of water held within the range between the two imbibitional pressures corresponding to the respective humidities. Thus if the soil sample lost a certain amount of weight while slowly changing from equilibrium conditions at 70 per cent. relative humidity to equilibrium conditions at 50 per cent. relative humidity, this loss would represent the amount of water absorbed with a pressure between 900 and 480 atmospheres.

Habitat measurements

By use of the methods here outlined one can calculate and compare the stromogenic tensions, and the imbibitional and osmotic pressures that could be developed in different environments, and at different times of day in the same environment. Thus in table II is shown the mean calculated stromogenic tensions for the months of January to July, 1932, at Washington, D. C.

The data are based on computations using Weather Bureau records[2] taken at 8:00 A.M., noon, and 8 P.M. Each value is based on the average relative humidity and temperature at the given hour for the entire month.

TABLE II

AVERAGE STROMOGENIC PRESSURES ATTAINABLE AT CERTAIN HOURS FOR THE FIRST SEVEN MONTHS OF 1932 AT WASHINGTON, D. C. DATA FROM WEATHER BUREAU

MONTH	8:00 A.M.	NOON	8:00 P.M.
	atm.	*atm.*	*atm.*
January	400	700	600
February	400	800	1000
March	500	900	800
April	600	1200	1000
May	500	1000	800
June	500	900	700
July	500	900	800

On the dryest day of January, 1932 (the 31st), these figures were, at 8:00 A.M., 1300 atm.; at noon, 1900; at 8:00 P.M., 1500.

Psychrometric readings taken among the dunes around Lake Michigan indicate occasionally relative humidities as low as 20 to 30 per cent., and an imbibitional pressure at such times equivalent to about 2000 atmospheres.

[2] The original meteorological data were furnished by Mr. M. C. BENNETT, Acting Chief of the Climatological Division of the Weather Bureau.

Laboratory air is usually arid, and seeds kept in laboratory storage will have a greater imbibitional pressure than seeds which are taken air dry from a natural environment. This is due to the greater evaporating tendency of water in laboratory air. The values found for seed colloids by SHULL (**6, 7**) using methods involving osmotic and vapor pressure equilibria, 950 to 1250 atmospheres, are therefore higher than the average values given in table II. The close agreement of the calculated imbibitional pressures required to prevent evaporation in natural atmospheres with the observed colloidal imbibitional pressures in arid dry seeds are in satisfactory agreement with the original measurements of these materials.

THE UNIVERSITY OF CHICAGO

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PREPARATION OF HUMATE IRON AND OTHER HUMATE METALS

C. KENNETH HORNER, DEAN BURK, AND SAM R. HOOVER

Introduction

It has been shown that humate iron is a highly active stimulator of bacterial growth and an excellent source of iron for the general nutrition of plants, and that it is often more convenient to employ than other forms of iron now available, such as the sulphate, citrate, or tartrate (**1, 2, 3, 6**). It is, moreover, not precipitated in physiologically alkaline solutions nor by high concentrations of phosphate. Our previous studies in this connection were not concerned with efficient methods of preparation of humate iron, since most of the work involved microcultures of bacteria and hence relatively small requirements of material. In view of the considerable interest in this form of iron, and other humate metals, which appears to have developed among plant physiologists, the conditions essential to obtaining the large amounts needed in the case of higher green plants have been worked out and are reported herewith.

Synthetic humate

In the preparation of the metal-free synthetic humate itself from sugar and hot strong mineral acid, the optimum percentage yield is confined to somewhat narrow ranges of the several determining variables. To obtain three-fourths or more of the optimum percentage yield, the following requirements hold in general: sugar, 3 to 20 gm. per 100 cc. acid solution; acid, 25 to 40 cc. conc. H_2SO_4 per 100 cc. acid solution; temperature and duration of heating, 2 to 3 hours at the boiling point (115° C.). The percentage yield decreases markedly above a sugar concentration of 20 gm. per 100 cc. because of greatly enhanced formation of caramel and humus compounds insoluble in dilute alkali. The absolute yield may be increased twofold as the sugar concentration is increased from 10 gm. per 100 cc. up to saturation, but the yield of secondary products is increased three to sixfold. Glucose, starch, sucrose, molasses, cellulose (filter paper), and sawdust give yields equivalent within 25 per cent.; dextrin and xylose appear somewhat inferior. Factors of cost, availability, and purity would determine the substance to be used. The writers have used sucrose and glucose chiefly. HCl is only about one-fourth as effective as H_2SO_4, at the optimum concentrations, and has the disadvantage of being volatile. Glacial H_3PO_4 is considerably less effective than HCl. Heating on the steam bath at 80° C. requires 48 hours to produce the yield obtained in 2 hours by heating over a flame or in an autoclave at 115° C. It is preferable to

employ volumes of at least 100 cc. of acid-sugar digestion mixture in containers several times this capacity. The iron or metal salt is no longer added to this mixture, as formerly, but to the finally purified neutral potassium humate, the details of preparation of which now follow.

One liter of 30 volume per cent. H_2SO_4 (*i.e.*, containing 300 cc. conc. H_2SO_4) is brought to the boiling temperature, 100–150 gm. sugar added, and the mixture allowed to simmer for 3 hours. It is then cooled and centrifuged in non-corrosive vessels, discarding the liquid phase. Concentrated KOH (or NaOH) solution is added to the solid phase until upon being partially dissolved the solution becomes alkaline to phenolphthalein, the latter being employed as an outside indicator and used with somewhat diluted portions of test solution. The material is recentrifuged, using either an ordinary centrifuge or a Sharples supercentrifuge. The solid phase contains alkali-insoluble humus and is discarded after one or more additional washings with dilute alkali to recover small amounts of occluded humate. The alkaline extract and washings, containing relatively pure, soluble potassium humate, are combined and further purified by at least one more cycle of precipitating with H_2SO_4 at pH 3 to 4 (brom phenol blue as outside indicator), centrifuging, discarding the slightly acid liquid phase, and dissolving the solid hydrogen humate by neutralization with 1 to 5 per cent. KOH to give a neutral or slightly alkaline solution (brom thymol blue employed as outside indicator). The concentration of humate in the final stock solution thus prepared may be determined by precipitating the humate in a 1–5 cc. aliquot portion in a weighed centrifuge cup, centrifuging, discarding the liquid, drying at 100°C., and reweighing. Occluded white K_2SO_4 sometimes present in the dried humic acid may be corrected for by washing with water, recentrifuging, and redrying as before, or by combusting the dried sample in a crucible and determining the ash. A yield of 15 to 30 gm. humate (10 to 20 per cent.) should be obtained from an original 150 gm. sugar.

A microcombustion carried out by MILDRED S. SHERMAN on synthetic humic acid prepared from glucose by this improved method and washed as free from ash as possible gave the composition ratio: C, 64.95; H, 3.85; ash (chiefly K_2SO_4), 0.5; and O (by difference), 30.7. This corresponds on an ash-free basis to an atomic ratio $C_{5.44}H_{3.84}O_{1.92}$, an empirical formula $(C_6(H_2O)_{2.1})_n$, and a molecular weight of $(109.9)_n$, where n is not necessarily a whole number. The synthetic humic acid is thus chiefly a condensation product of sugar, with the loss of about two-thirds of the molecular water. Little or no oxidation is involved, unless of a Cannizzaro or similar internally compensating type. Methoxyl groups ($-OCH_3$) occur.

The theoretical yield of humic acid from glucose calculates out as (109.9/180.1)(100), or 61.0 per cent., and is considerably less than 100 per

cent. because of the much lower molecular weight of humic acid per unit of carbon. The experimental yield of 10 to 20 per cent. is thus about one-fourth the maximum theoretically possible. In view of the numerous side-reactions occurring, this yield, while conceivably still improvable, must be regarded as satisfactory, especially as it represents an improvement of 50 to 100-fold over the formerly obtained 0.2 per cent.

The preparation of synthetic humate from sugar has been studied to a certain extent by FRAPS (**5**). No separation was made of the large amount of charred carbon and other alkali-insoluble and acid-insoluble humus material from the acid-insoluble, alkali-soluble humic acid proper, and hence the results obtained were not comparable with those presented here. Much of the basic chemistry and methods of preparation of both the synthetic and natural humates was already well worked out one hundred years ago by SPRENGEL (**7**), but not from the detailed point of view of interest and applicability here presented.

Natural humate

Natural humate is extracted from soils as before (**1,** p. 416), except that only one or two cycles of purification are strictly necessary, instead of four or five as then recommended. Garden soils should yield 1 to 4 gm. humate per 100 gm. soil, and more in the case of peat. Any naturally decomposed organic matter may be employed. Natural humate differs from synthetic in consisting of a large number of organic compounds and inorganic elements, and also nitrogen. It is not yet known how many, if any, of the organic compounds are identical in both cases, but close relationships with respect to certain of the fundamental and important properties are definitely involved. The iron content of natural humate will vary from about 0.1 to 1.5 per cent. as the HCl-washing preliminary to the alkali extraction (**1,** p. 416) is varied from 0 to 24 hours; the iron is derived from the soil substances.

Humate iron and iron humate

In preparing humate iron, an inorganic iron salt is added directly to a neutral solution of potassium humate of known humate concentration. A concentrated solution such as 10 or 20 per cent. $Fe_2(SO_4)_3.9H_2O$ is convenient to employ. All preparations of natural or synthetic humates so far obtained have taken up at room temperatures at least 10 per cent. by weight of Fe, generally 15 per cent., and at times 20 to 25 per cent. The maximum percentage of iron held in solution by humate is substantially independent of the concentration of humate in the stock solution, at least over the 50-fold range of interest in connection with stock solutions, namely, 1 to 50 mg. per cc. It is generally desirable in the case of any particular humate to determine the maximum Fe/humate ratio in a small aliquot portion, and

then add iron to the main bulk of the stock solution to give a ratio 10–50 per cent. smaller.

Ten to 20 per cent. Fe in the soluble potassium-humate-iron preparation approximates the proportion of iron in the ordinary iron sulphate, tartrate, or citrate, but the manner of combination appears to be definitely different, hence *the term humate iron has been tentatively employed for iron thus held in solution, as compared with the compound iron humate highly insoluble in water.* Adsorption and colloidal states may be involved, but a basic substance is formed in the chemical sense that an amount of strong alkali approximately equivalent to complete neutralization of the potential acidity of the iron salt, with the formation of an alkali salt and the base ferric hydroxide, is necessary for the complete and stable solution of the iron in the neutral potassium humate solution. Humic acid itself, moreover, is not to be placed in the same category with simple organic acids.

The potential acidity of the iron salt added to the humate solution lowers the pH, and a precipitate of chiefly iron humate and some hydrogen humate is formed which is brought into solution again by further addition of strong alkali. The volume of solution is made up to some suitable value and the concentrations of humate and iron recalculated. Convenient concentrations for stock solutions are 2 mg. Fe and 15–25 mg. humate per cc., so that an initial dosage of 1 cc. per liter of nutrient medium provides 2 p.p.m. Fe, which, without further replenishment, should carry most bacterial cultures or green plants well toward maturity. Stock solutions will last indefinitely without further significant change. If desired, iron-hydrogen humate, obtained by precipitation of humate iron at pH 3 or less, centrifugation, and decantation, may be dried, so stored, and later dissolved with alkali as needed. If some chemical supply-house were to prepare this material and place it on the market for the benefit of investigators of nutritional problems, the solid form would probably be preferable, as in the case of many comparable indicators and dyes.

The formation of insoluble iron or hydrogen humate is not essential to the process of humate iron stabilization, as can be shown by making the potassium humate solution appropriately alkaline with KOH before the iron salt is added, so that upon mixing the two clear solutions the pH immediately assumes an equilibrium value between 7 and 9; no precipitate is observed to form unless momentarily due to insufficiently rapid mixing. Above pH 10 the maximum amount of iron kept in solution by a given amount of humate becomes somewhat reduced, the more so as the alkalinity is increased. In this same connection, a very great initial alkalinity of the humate solution will precipitate ferric hydroxide and greatly retard the rate and amount of formation of soluble humate iron.

A ferrous salt, if employed in place of a ferric salt in the preparation

of neutral humate iron, is oxidized completely to the ferric state by atmospheric oxygen in a few minutes, as we have found with the Warburg manometric apparatus. Hence there appears to be no advantage in employing ferrous rather than ferric iron. The ferrous salt does not reduce the humate appreciably during its oxidation, the rate and extent of which by oxygen gas are substantially the same in the presence and absence of humate, the pH being kept constant and either alkaline or very mildly acid.

Attention should be called to the fact that both natural and synthetic alkali humates are subject to precipitation by relatively high concentrations of one particular element found in many plant nutrient media, namely, calcium. Fortunately the lowest concentration of soluble calcium generally capable of causing significant precipitation, about 0.01 M, is far above that employed in bacterial media (0.001 M or less) and somewhat above that employed in the great majority of green plant nutrient media (0.002 to 0.005 M). All humates appear to be completely precipitated at 0.01 to 0.05 M Ca. The actual extent of precipitation depends chiefly upon the given concentration of calcium, but also somewhat upon the percentage of iron or metal attached to the humate, the particular humate preparation involved, and the concentration of humate. It has been observed empirically that the greater the capacity of a given humate to hold iron in solution (as humate iron) the greater the concentration of calcium required to cause humate precipitation (as calcium or iron humate). Certain plant nutrient media containing high concentrations of calcium may be found to precipitate some of the humate in particular humate preparations. In these cases it may be practicable, and not disadvantageous, to lower the calcium concentration to 0.003 M. It may also be desirable to decrease the ratio of iron to humate in the humate iron preparation. Magnesium precipitates humates only at concentrations five to ten times those required in the case of calcium. Precipitated calcium humate is readily soluble in alkali hydroxide so long as the concentration of free calcium ion is low, about 0.01–0.02 M. When for any reason the calcium ion concentration is considerably higher than this value, it may be reduced below it by addition of oxalate or some similar substance which precipitates calcium ion, and any precipitated calcium humate originally present will then go into solution upon addition of any necessary KOH. This circumstance is very fortunate in the process of extracting natural humate from highly calcareous soils, or from decomposed filter press cake, when for some reason these substances are first acidified and partially dissolved. The latter, a by-product of sugar manufacture, contains a high percentage of excellent natural humate, but also a high percentage of calcium, which with acidification would ordinarily prevent satisfactory solution of the humate in alkali, and would, in the absence of oxalate treatment, have first to be removed with great difficulty by means of extended acid washings.

Humate metals

Humate iron is only one member of a large class of similar humate metals. Al, Cu, Zn, Ni, Co, Hg, Ti, Cr, Mn, etc., all form hydroxides insoluble somewhere over the physiological pH range 3 to 9. Salts of these metals added in not too great excess lower the pH of a neutral potassium humate solution with the formation of more or less precipitated metal humate. These metal humates are dissolved upon further addition of alkali to give soluble, approximately neutral, humate metals. The maximum percentage of soluble metal taken up in solution by humate is about the same for each metal, 5 to 20 per cent. by weight of the humate. This maximum percentage is practically independent of the concentration of humate. It represents, moreover, the maximum for the total metal held in solution when several metals are added simultaneously, *i.e.*, the metals compete with one another for maintenance in solution. When metal is added just exceeding the maximum percentage, the insoluble hydroxide, and not the soluble basic humate, of the excess metal is formed upon making the pH neutral with alkali. But if the excess is several times greater than the amount held in solution, the whole of the humate is eventually precipitated in a form not easily dissolved even in strong alkali. The humate insolubility in this case depends markedly upon the initial humate concentration. In all these phenomena the various metals named behave like iron. It is obvious that in forming stock solutions care must be taken not to add too great an excess of metal, or some metal hydroxide or even metal humate will occur as a precipitate in the stock solution brought to neutrality. ERDENBRECHER (**4**) has described somewhat similar humate metal preparations.

A small class of metals, including Ca, Ba, Mg, etc., exists whose salts do not greatly alter the pH of a neutral potassium humate solution and whose hydroxides are relatively soluble over the entire physiological range. The ions of these metals form, when added to potassium humate solution in sufficient concentration, precipitated metal humates (*e.g.*, calcium humate, magnesium humate) which are also very difficultly soluble in strong alkali. The precipitating concentration depends here also markedly upon the concentration of humate as well as of metal. In a sense these metals may be considered as destroying the colloidal condition of the humate.

Natural humate, containing as it doubtless does traces of practically every element of significance in the growth of green plants—and in quite possibly highly available form—would appear to be a somewhat ideal substance to provide so-called "shot-gun" nutrition, especially when enriched with 5 to 15 per cent. iron and supplied as 10 to 20 parts per million of humate (or more), so as to provide iron at a concentration of a few parts per million and traces of the other elements at thousandths to tenths of a part per million. This iron-enriched natural humate could well be used in most culture work where the purpose is to obtain good healthy plant

growth in media varied in connections other than traces of elements. The use of the synthetic humate, which is somewhat more difficult and expensive to prepare, could be reserved for cases where it is desired to study the effect of intentionally added traces of specific elements and where application of the natural humate would obviously be undesirable because of the possibility of its containing these traces in significant amounts.

Summary

1. Efficient and detailed methods have been worked out for the preparation of synthetic and natural humates containing iron, aluminum, manganese, zinc, nickel, copper, or other similar metals whose inorganic salts are ordinarily highly insoluble over some portion of the physiological range of pH of interest in connection with plant growth.

2. One or two small applications of these humates will ordinarily supply sufficient iron or similar metal to water or sand cultures to last throughout the entire period of growth. The metals in the form of humates are stable in alkaline and neutral as well as moderately acid culture media, and are not precipitated by phosphate.

FERTILIZER INVESTIGATIONS UNIT
BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

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CHEMICAL CHANGES IN CARROTS DURING GROWTH

HANS PLATENIUS

(WITH FIVE FIGURES)

Introduction

This study was carried out in connection with a general investigation of the factors influencing quality in carrots. It was recognized that age and size of the roots play an important rôle in determining quality. Although it was realized that quality in vegetables, an indefinite term in itself, cannot be measured accurately, it was expected that the analysis for certain chemical constituents would give some indication of changes in eating quality as well as in nutritional value of the carrots.

Since it was necessary to take samples at various stages of the growth period, the plan of the experiment was enlarged so as to gain some information regarding the rate of growth of the plant, the rate at which certain mineral nutrients are absorbed, and various products of photosynthesis are formed.

Materials and methods

The carrots for this study were grown in the gardens of the Department of Vegetable Crops at Cornell University during the summer of 1932. Manure and a 5–10–5 fertilizer were applied to the soil prior to sowing. An overhead irrigation system supplied adequate moisture to the plants during periods of drought. Seed of the Chantenay variety was sown May 1 in rows 18 inches apart. About August 15 the carrots had attained the optimum size for market. Growth of the roots continued until the last day of sampling, October 15, but they were then of such a large size that they would have been considered of inferior quality on the market.

SAMPLING

The first sample was taken June 16, one and a half months after sowing of the seed. Between June 16 and August 15 the carrots were sampled at approximately semi-monthly intervals; after August 15 at monthly intervals. A representative sample of at least twenty individual plants was taken at noon of each sampling day and an aliquot of the composite sample was later used for chemical analysis. The whole plant with the exception of the smaller side roots was pulled and taken at once to the laboratory. Roots and tops were separated and the weight of each was recorded. The material from both lots was then cut into small pieces and mixed thoroughly. An aliquot of 100 gm. was used for the determination of dry weight while another aliquot of 150 gm. was dropped into jars containing sufficient 95

per cent. alcohol to make a final concentration of about 75 per cent. After boiling for 10 minutes the jars were sealed and stored for analysis.

Methods of chemical analysis

(a) Dry weight.—The material was dried in a ventilated oven at 60° C. for 40 hours after the sample had been heated at 100° for one hour to inactivate the enzymes.

(b) Sugars.—A modification of the picric acid reduction method described by Willaman and Davison (**8**) was used.

(c) Starch.—The extracted material was digested with takadiastase at 38° C. for 20 hours, filtered, and the filtrate clarified with lead acetate, deleaded, and hydrolyzed with dilute hydrochloric acid. Glucose was determined in the hydrolyzed material by the picric reduction method.

(d) Crude fiber.—A slightly modified official procedure was used.

(e) Lignin.—A method which was described in detail by Phillips (**6**) was used. Lignin was determined gravimetrically after cellulose had been removed by fuming hydrochloric acid. Corrections were made for ash and protein.

(f) Calcium and phosphorus.—These constituents were determined by the official method recommended by the A.O.A.C. (**5**).

Analytical results

The results of the chemical analyses were expressed as percentages of dry weight, which made it possible to eliminate certain fluctuations in composition caused by variations in the moisture content of the plant. In order to study the relationship between the rate of formation of the various chem-

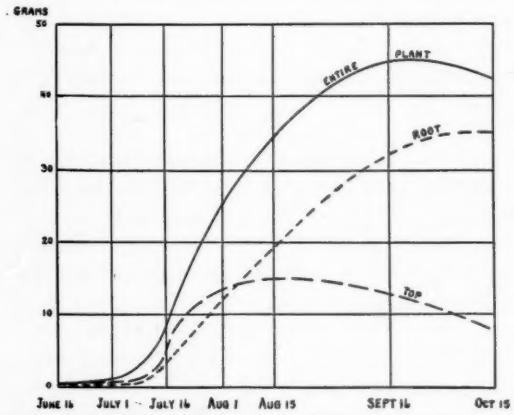

Fig. 1. Rate of growth of entire carrot plant, root, and top in grams of dry weight.

ical constituents analyzed, the data were also calculated as weight per plant or root respectively. A graphic representation of the rate of growth of the root, top, and entire plant is given in figure 1. The various curves do not give a clear conception of the true relative growth rate; a better representation can be obtained when the rate of growth is plotted on a ratio scale (fig. 2), where the paper in the vertical direction is ruled on a logarithmic

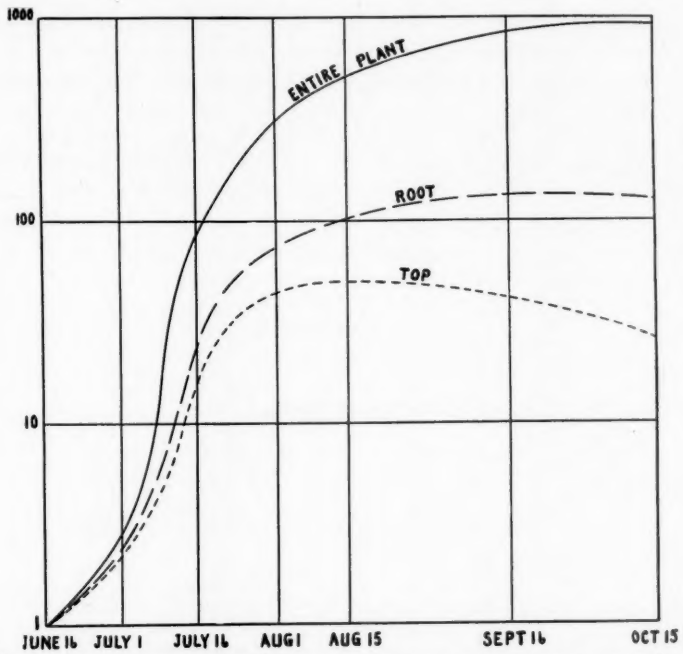

FIG. 2. Rate of growth of entire carrot plant, root, and top. Data (calculated on dry weight basis) are the same as given in fig. 1, but are plotted on the ratio scale.

scale while the horizontal scale gives time in absolute amounts. The nature of the ratio scale is discussed in detail by CHADDOCK (**2**). The spacings in the vertical direction represent true ratios, the slope of the curve indicates the rate of increase or decrease, and the slopes of different curves are comparable and represent comparative rates of increases or decreases regardless of the absolute values. Only the slopes are comparable, not the levels of the points above the base line. The obvious advantage of using such a scale is to bring several curves close together for comparison and to show the true relationship between relative increases or decreases. The ratio scale has been used by BUCHANAN and FULMER (**1**) to illustrate the growth rate

of bacteria. Strangely enough, few other workers dealing with growth rates of plants have used this method of presentation.

A few striking facts become apparent when the chemical composition of the root at the various stages of growth is examined with regard to those constituents which ordinarily are considered as factors determining quality.

The moisture content fluctuated somewhat and decreased slightly with age. The decrease in the water content was not more than 2.5 per cent. of the entire fresh weight, however, and with an average moisture content of nearly 90 per cent. it becomes doubtful whether such small fluctuations can materially influence the crispness of the tissue as far as eating quality is concerned.

The total sugar content increased slowly, and on the dry weight basis was about 2.5 per cent. higher in the old carrots than in the young ones (table I and fig. 3). A very definite relation was found between reducing

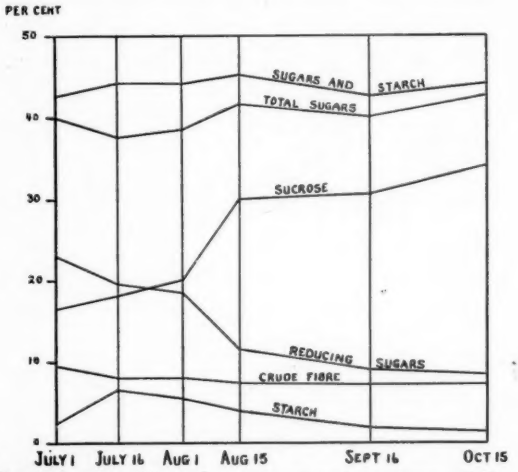

FIG. 3. Chemical composition of carrots at various stages of the growth period. Data expressed as percentages of dry weight.

sugars and sucrose. While the reducing sugars declined steadily, sucrose increased at about the same rate. On a percentage basis reducing sugars dropped to less than one-half of their original value while the percentage of sucrose more than doubled. In order to determine the relative sweetness of carrots at different stages of age it is important to identify the reducing sugars present, since different sugars vary widely in their relative sweetening power. For instance, should the greater part of the reducing sugars be in the form of fructose, young carrots would be sweeter than old ones. On the other hand, if glucose forms most of the reducing sugars the relation

TABLE I

CHEMICAL COMPOSITION OF CARROT ROOTS AND TOPS AT VARIOUS STAGES OF GROWTH. ALL DATA EXPRESSED ON DRY WEIGHT BASIS

DATE	DRY WEIGHT	AVERAGE WEIGHT OF PLANT	SOLUBLE NITROGEN	PROTEIN NITROGEN	TOTAL NITROGEN	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	CRUDE FIBER	LIGNIN	CaO	P_2O_5
	%	*gm.*	%	%	%	%	%	%	%	%	%	%	%
ROOTS													
June 16	10.04	0.037											
July 1	11.34	0.1066	0.580	1.234	1.814	23.24	16.53	39.77	2.52	9.50	2.98	0.066	1.356
July 16	12.42	3.116	0.436	0.914	1.350	19.32	18.20	37.52	6.62	7.94	2.81	0.094	0.919
Aug. 1	11.12	11.70	0.612	0.803	1.415	18.52	20.05	38.57	5.47	7.83	2.95	0.078	0.933
Aug. 15	11.38	19.17	0.481	0.861	1.342	11.53	29.82	41.35	3.89	7.63	2.49	0.080	1.004
Sept. 16	12.50	32.11	0.674	0.786	1.460	9.86	30.36	40.22	2.05	7.24	1.83	0.069	1.121
Oct. 15	12.44	34.82	0.621	0.766	1.387	8.46	33.91	42.37	1.48	7.30	1.95	0.061	1.031
TOPS													
June 16	14.17	0.2996											
July 1	16.53	0.6771	0.471	0.309	0.780	2.81	5.34	8.15	0	9.04	5.18	3.37	1.158
July 16	16.13	4.655	0.286	0.261	0.547	6.57	1.48	8.05	0	10.60	6.82	3.36	0.664
Aug. 1	16.95	13.57	0.233	0.189	0.412	7.91	1.99	9.90	0	11.03	6.50	3.23	0.778
Aug. 15	17.62	15.14	0.174	0.198	0.372	6.87	1.52	8.39	0	10.47	8.13	3.59	0.724
Sept. 16	19.39	13.04	0.158	0.224	0.382	3.12	1.70	4.82	0	12.24	11.21	4.93	0.683
Oct. 15	21.96	7.879	0.170	0.208	0.378	2.70	1.53	4.23	0	13.44	10.73	4.57	0.673

would be reversed. The isolation and identification of certain sugars in a mixture is a difficult procedure. PINOFF and GUDE (7) describe a color test using ammonium molybdate, which is fairly specific for fructose. Using this test, only traces of fructose could be found in the mixture of sugars extracted from young carrot roots. Assuming that all reducing sugar is present as glucose, older carrots should be 19 per cent. sweeter than young ones when the relative amounts of these sugars present is considered. This agrees very well with the fact that old carrots were found to be noticeably sweeter to taste than young roots. This fact conforms with the statement of HASSELBRING (3) who considers the natural content of sucrose as determining largely the flavor in carrots.

The percentage of starch increased very rapidly at first to 6.6 per cent., then declined slowly to 1.5 per cent. of the dry weight.

Very surprising is the fact that the amount of crude fiber, which was expected to increase with age, actually declined. The same holds true for lignin, which occurs in small amounts particularly around the tracheae.

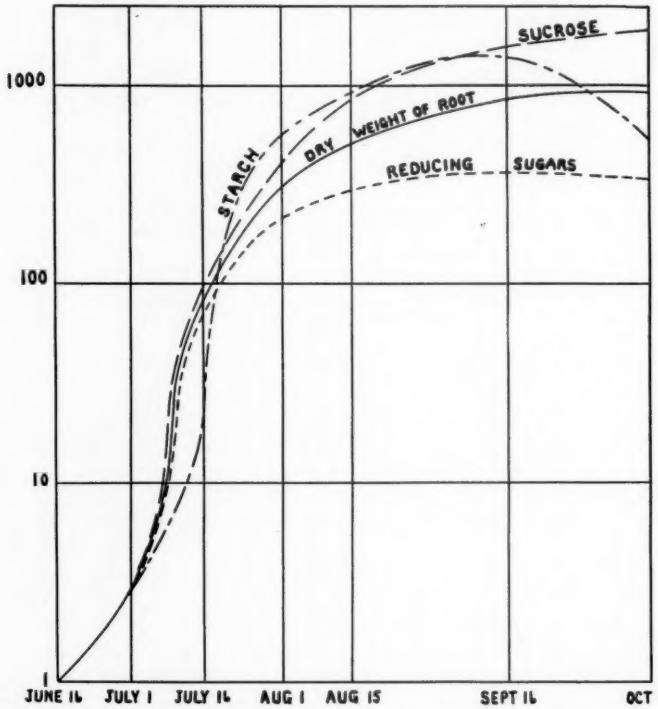

FIG. 4. Rate of accumulation of carbohydrates in carrot roots. Data plotted on ratio scale.

When the growth curves for the roots and tops are considered separately it is found that their rates of growth differ widely (fig. 2). While the tops had ceased growing entirely August 15, the roots were still gaining in weight two months later. The tops actually lost some of their dry matter after August 15. This loss was partly due to respiration and the slow dying off of the outer leaves. It can safely be assumed, however, that the principal loss was due to translocation of sugars from the leaves to the roots. In this connection it is interesting to notice that no trace of starch was found in the leaves at any time.

Figure 4 shows that reducing sugars accumulated in the roots at a much slower rate than sucrose, which necessarily resulted in a much lower percentage of glucose than sucrose at the end of the storage period. The formation of starch in the roots was most rapid during July, the relative rate of increase being greater than that of the total dry weight or of the sucrose content in the roots in this period. During the last month, from September 15 to October 15, there was a sharp drop in the starch content. Apparently, the starch was reconverted into sucrose, which continued to accumulate until the last day of sampling.

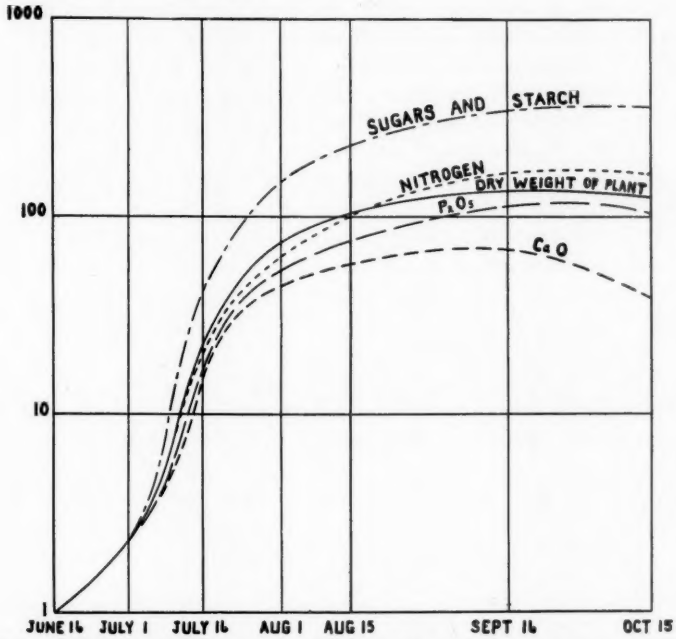

FIG. 5. Rate of absorption of calcium, phosphorus, and nitrogen and rate of accumulation of starch and sugars in the entire plant. Data plotted on ratio scale.

In figure 5 is shown the relative rate of the absorption of some mineral constituents, calcium, phosphorus, and nitrogen, by the entire plant. It is obvious that the relative rate of absorption of minerals is much greater at the beginning of the growth period than at the end when compared with the accumulation of photosynthetic products. During the last stage some of the mineral and nitrogen constituents actually disappeared from the plant, probably through the dying off of the outer leaves. It is doubtful whether any minerals actually migrated out of the plant through the roots. The more rapid loss in calcium as compared with the loss in phosphorus or nitrogen is explained by the fact that the tops contained four to seven times as much calcium as the roots, while phosphorus and nitrogen were distributed more evenly through the entire plant.

Discussion

There is a general assumption among consumers that small young carrots are far superior to large old ones, which is reflected by the fact that young carrots bring a much higher price on the market. The present study makes it extremely doubtful whether this assumption is justified. The analytical data show clearly that sweetness in carrots, which probably is controlled primarily by their sucrose content, increases markedly with age.

In the present study no data were obtained which support the general idea that young carrots are more tender than old ones. On the contrary, the amount of crude fiber was found to decrease slightly with age. Several persons in the Department of Vegetable Crops, Cornell University, who compared young and old carrots with respect to tenderness, agreed that no significant difference could be observed between the two groups. If a slight difference was noticed it was always in favor of the old ones. Chemically and microscopically no change in the amount of lignified matter could be found. In older carrots, however, the cells of the outermost cortex are very much smaller, which brings the cell walls closer together and consequently the skin is slightly harder than in young carrots. Under certain conditions it may be found that young carrots are more crisp than old ones, especially if the latter group has been kept in storage for some time. Crispness is controlled by the water content of the roots and the turgidity of the cells.

The color of the carrots as determined by the amount of carotene present improved decidedly with age. Although no measurements of the relative amounts of carotene present were made, the difference in color was striking and consistent. Since it is a generally accepted theory that carotene and vitamin A content are closely connected, this would suggest the possibility that the vitamin A content in old carrots is higher than in young ones. The percentage of dry matter and combined sugars and starch was shown to

increase slightly with age while the amount of crude fiber decreases. This indicates that the food value of carrots tends to increase rather than decrease with age.

HASSELBRING (3) concluded from his data that starch is absent in carrots, although he cited the work of other investigators who reported its presence. The writer was able to verify the occurrence of starch by identification of starch grains in the dried root tissue.

The curves obtained for the rate of growth and the rate of accumulation of photosynthetic products are extremely smooth, especially if it is realized that this experiment was carried out in the field under variable weather conditions.

The rôle which the root plays as a storage organ is well illustrated. For two months after the tops had ceased growing, translocation of carbohydrates from the tops to the roots continued.

Little work has been done on the rate at which mineral constituents are absorbed by vegetables. Such studies may be of importance in determining the proper time at which fertilizer can be applied to the plants to best advantage. KOTOWSKI (4) made such a study for several vegetables. However, he took only three samples during the entire growing period, and this can hardly be considered sufficient to give accurate data on the rate at which nutrients are absorbed. The present results show that all minerals had been absorbed by the plant at least a month before the roots obtained their maximum size.

Summary

1. Data are given which indicate that carrots lose neither in eating quality nor in food value as they grow older. The sucrose content, which is considered to determine flavor in carrots, rises decidedly while the percentage of crude fiber becomes slightly lower.

2. Older carrots are better colored owing to their higher carotene content.

3. The occurrence of small amounts of starch in carrot roots was verified. The tops were found to be free of starch throughout the growth period. Curves are presented of the growth rate of roots and tops and the rate of accumulation of various chemical constituents in the plant.

CORNELL UNIVERSITY
ITHACA, NEW YORK

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APPLICATION OF QUANTITATIVE SPECTRAL ANALYSES TO BINARY MIXTURES OF THE COMMON CAROTENOIDS

ELMER S. MILLER[1]

The application of quantitative spectral analyses to biological compounds was first reported by KUHN and SMAKULA (**1**) in 1931. By trial and error, they varied the amounts of each component in solution until the resultant absorption curve was identical with that obtained for the unknown mixture. At best, this method is limited in its application. The accurate spectro-photoelectric method described by ZSCHEILE, HOGNESS, and YOUNG (**2**) has been used to obtain the quantitative data presented in this paper.

From Lambert's law, we obtain this relationship[2] (ZSCHEILE, **3**):

$$\log \frac{I_o}{I_x} = \alpha c\, x \qquad (1)$$

When two components are present, the *effective* absorption coefficient β_T (for the total number of components) is defined by the following consideration:

Let:

$$\beta_1 = \alpha_1 c_1 \text{ and } \beta_2 = \alpha_2 c_2$$

$$c_T = c_1 + c_2 \text{ (the total concentration)} \qquad (2)$$

and

$$\beta_T = \alpha_T\ c_T \qquad (3)$$

then

$$\beta_T = \beta_1 + \beta_2 = \alpha_1 c_1 + \alpha_2 c_2 = \alpha_T\ (c_1 + c_2) \qquad (4)$$

From ZSCHEILE (**3**)

$$\log \frac{I_o}{I_x} = \beta_T x = \alpha_T c_T x \qquad (5)$$

If for some wave length, λ', $\alpha_1' = \alpha_2'$

[1] National Research Fellow in the Biological Sciences.

[2] In the equations superscripts refer to wave lengths and subscripts to the different components, and

I_o = intensity of light transmitted by solvent-filled cell.
I_x = intensity of light transmitted by solution-filled cell.
x = thickness of absorption cell (4.25 cm.).
β = absorption coefficient.
α = specific absorption coefficient.
c = concentration in gm. per liter.

then

$$\beta_T' = \alpha_1' (c_1 + c_2) \qquad (6)$$

Thus when β_T is measured at λ', and α_1 and α_2 are known, c_T may be calculated.

The relative and absolute amounts of components 1 and 2 can be determined by measurement of β_T'' for some other suitable wave length λ'' for which there is an optimum difference between α_1 and α_2; thus

$$\beta_T'' = \alpha_1'' c_1 + \alpha_2'' c_2 \qquad (7)$$

Hence, c_1 and c_2 can be determined by solving equation (2) and equation (7).

It is not necessary to use a wave length for which $\alpha_1 = \alpha_2$. In general, c_1 and c_2 can be determined by solving simultaneously two equations like (7) obtained for two suitable wave lengths.

Accurate analyses of binary mixtures of the carotenoids

Before the analyses of binary mixtures can be undertaken, it is necessary to determine the absorption coefficients of the pure components (fig. 1, Plant Physiol. **9**: 693. 1934). These data permit the selection of the suitable wave lengths of light to be employed in the analyses.

Since the total concentration of the mixtures was known (1.2 mg./L.), β_T was not measured at a wave length at which components 1 and 2 absorb equally. In figure 1,[3] it is seen that both the carotene isomers absorb equally at 4450 Å. U. Hence, from experimentally determined values of β_T at this wave length, it is possible to calculate the total concentration $(c_1 + c_2)$ by equation (6). The percentage composition of each component present can then be determined by the measurement of β_T'' (equation 7). For the purpose of checking upon each other, two or more wave lengths were employed. The standard solutions were made up with an accuracy of less than 1 per cent. error.

The effective absorption coefficient, α_T, for known mixtures was plotted against compositions. This gives a straight line relationship which may be used in the analyses of unknowns (ZSCHEILE, **3**). This straight line relationship also indicates that Beer's law holds for the concentration employed.

α_T is obtained from measurements for which equation (6) is employed. The following equation (8) shows analytically that such a relationship $\left(\frac{c_1}{c_1 + c_2}\right)$ should exist. From equation (4) we obtain,

$$\alpha_T = \alpha_1 \frac{c_1}{c_1 + c_2} + \alpha_2 \frac{c_2}{c_1 + c_2} = \alpha_1 \frac{c_1}{c_1 + c_2} + \alpha_2 \left(1 - \frac{c_1}{c_1 + c_2}\right)$$

[3] In this study, the same solvent was employed as in Plant Physiol. **9**: 693–694. 1934, and reference 4.

or

$$a_T = (a_1 - a_2)\frac{c_1}{c_1 + c_2} + a_2 \qquad (8)$$

The percentage composition of mixtures of alpha and beta carotene, beta carotene and lycopene, and lycopene and leaf xanthophyll were calculated from the experimental data at the various wave lengths by employing equation (8) and the final values of a_1, and a_2 (of the pure components), as presented in fig. 1. The percentage composition for the binary mixtures of the carotenes are summarized in Table 1. The greatest deviation from the true composition in any mentioned binary mixture is 0.8 per cent. and the accuracy of this method may be seen by a comparison of the first and last rows of figures.

TABLE I

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF ALPHA AND BETA CAROTENE IN 20.0 PER CENT. ETHER AND 80.0 PER CENT. ETHANOL

		Composition in terms of beta carotene								
Known composition (per cent.)		5.0	10.0	25.0	50.0	75.0	90.0	95.0	Extract of analyses in samples	
	Wave length	%	%	%	%	%	%	%	*I*	*II*
Quantitative	4636 A. U.	5.3	9.8	25.2	50.2	75.2	89.9	94.5	69.2	69.3
spectral	4862 A. U.	4.8	9.9	24.9	49.8	74.8	90.2	95.2	69.7	69.1
analyses	4956 A. U.	5.4	10.1	25.1	50.0	74.8	90.3	94.8	67.5	68.2
Av. per cent. composition		5.2	9.9	25.1	50.0	74.9	90.1	94.8	68.8	68.9

Analyses of unknowns

Columns I and II in table I contain the analyses of solutions of unknown composition. Determinations for the three wave lengths agree within 1 per cent. error. This method is now employed for the quantitative analyses of the carotenoids present in the grasses with about the same degree of accuracy. Thus, by this spectro-photoelectric method, it is possible to analyze unknown binary mixtures of the carotenoids and to determine the total concentration and the percentage composition with an error of about 1.0 per cent.

Summary

1. Quantitative spectral analyses have been applied to binary mixtures of the common carotenoids. The percentage composition of binary mixtures of the carotenoids were determined with an error of about 1 per cent.

2. Over a limited range of concentration, it has been shown that Beer's law is obeyed by binary mixtures of carotenoids.

The writer wishes to express his appreciation to Prof. T. R. HOGNESS for suggestions and invaluable criticism during the progress of this investigation, and to Dr. F. P. ZSCHEILE for his valuable criticism of the paper.

GEORGE HERBERT JONES CHEMICAL LABORATORY
THE UNIVERSITY OF CHICAGO

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WHAT THE ORGANIC CHEMIST CAN DO FOR PLANT PHYSIOLOGY[1]

HUBERT BRADFORD VICKERY

It is not customary for addresses made to scientific gatherings to be founded upon a text after the manner of a sermon. Notwithstanding this, I am going to use a text on the present occasion. A few years ago a valuable little book appeared entitled "Recent Advances in Plant Physiology," written by E. C. BARTON-WRIGHT, of King's College, London.[2] In the preface to the book the author makes the following statement:

"Our present knowledge of plant metabolism is very seriously deficient in several directions. This unfortunate state of affairs is due to several causes, among the principal of which is the fact that in plants several complex chemical reactions take place within the compass of a single cell, which makes the matter difficult of investigation. Nevertheless, the difficulty is intensified by the sporadic invasions of organic chemists into a domain of which they have little or no knowledge, with ready-made explanations based on *in vitro* experiments which are probably remote from the chemical reactions of the living plant. It is difficult to know why botanical physiology should be made the general playground for the imaginative theorising of persons who have no very reliable knowledge of the living plant."

It seems to me that the occasion of a STEPHEN HALES address by one who is frankly a chemist, and who certainly lays no claim to a "reliable knowledge of the living plant" presents an opportunity for a defense of the custom imputed to us chemists of straying into places where we do not belong, and where apparently we are not universally welcomed.

Has the organic chemist anything to offer to plant physiology that entitles him to expect a welcome? One of the greatest of recent plant physiologists, evidently believes that he has. The late KOSTYCHEV writes,[3] "Physiology endeavors to trace the phenomena of life to the general laws of chemistry and physics, *i.e.*, in the final analysis, to state all the chemical processes in organisms by chemical equations, and to interpret the energy transformations and the entire structural features on the basis of physical laws."

Let us consider this view of the aims of physiology in a little detail. The plant absorbs its nutriment from the soil in the form of an aqueous solution of inorganic ions, the most important being nitrate, ammonium, phosphate,

[1] Third STEPHEN HALES address, read at the Boston meeting, December 29, 1933.

[2] BARTON-WRIGHT, E. C. Recent advances in plant physiology. Blakiston. Philadelphia. 1930. (p. vi.)

[3] KOSTYCHEV, S. Chemical plant physiology, translated by C. J. LYON. Blakiston. Philadelphia. 1931. (p. xiii.)

and potassium, with magnesium, calcium and a number of others of less significance only inasmuch as they are needed in smaller amount. Its carbon it takes directly from the air as carbon dioxide. From these extremely simple components the cells manufacture all that is required for the growth and reproduction of a highly complex organism. No one can state the number or the identity of the organic substances in a plant cell. We know in a general sort of way that the cell contains proteins, carbohydrates, fats, lecithins, sterols, hydrocarbons, hydroxy-acids, amino-acids, peptides, amides, purines, betaines, quaternary bases such as choline, to mention some of the better recognized types of substances; in addition there are enzymes —substances probably of protein nature which act as catalysts of the myriads of reactions that occur—and inorganic salts.

As I see it there are two main groups of plant physiologists—those who are chiefly interested in such topics as plant movements in response to various stimuli, in the response of the plant to changes in illumination, or of temperature, or of the composition of the air surrounding it, or of the soil solution that bathes its root; in short those who are concerned with the behavior of the plant unit as a living organism. But there are also many who are interested in the detailed problems of plant function, in the means by which it absorbs its food, and what it does with it, in the mechanism whereby it transports the elaborated products derived from the food substances, in its capacity for food storage and subsequent utilization, in its response to adversity, or injury. Such people have an insatiable curiosity to discover "how it works," and it is to these that the chemist can render his chief service.

I wish to discuss two of the functions that the chemist fulfils for these persons. He can supply them with methods, and he can supply them with critique—both of these functions are of far-reaching importance.

Measurement is a primary requisite to real advance in knowledge. The human mind responds most eagerly when a statement is couched in quantitative terms. We may be interested to learn that business is better, but the information that the bank clearings have increased by such and such a sum during the past week is far more meaningful. It is of more significance to state that a leaf, when placed in water, has increased in weight by such and such a per cent., than to state that it has become more turgid.

The function of the chemist is to supply methods whereby accurate measurements of the composition of tissues can be made. It is his responsibility to see to it that his method shall be specific and, where this is difficult or impossible, to provide for controls and corrections whereby the effect of interfering substances is minimized. It is his further responsibility to see to it that his method is accurate and reliable. In addition it is thoroughly desirable that his method shall be rapid and convenient. Methods that combine

all three features of specificity, reliability and convenience will occur to all of you. Some of these methods, such as the Kjeldahl method, have become integral parts of the equipment of every laboratory; others, such as the quinhydrone method for hydrogen-ion activity measurements, are now very commonly employed, while still others, such as an extensive group of colorimetric methods which require the use of a spectrophotometer, are only beginning to make their way into physiological laboratories. Many methods require the development of special equipment for their convenient application. This has certain disadvantages, especially when funds are limited, but the psychological effect is usually good. One approaches a piece of new apparatus with a species of awe and, after he has turned a stop-cock at the wrong moment a few times, this may change to annoyance; but finally, when he has compelled that inanimate object to tell him the truth again and again, his feeling becomes tinged with respect, and he acquires a sense of mastery over nature that is not without its own peculiar reward.

This brings me to another point. One may develop a method that has all desirable features, and describe it in full detail, but no control can be exercised over what the users of the method may do with it. There is probably no method so simple that it does not have to be learned by repeated trial. Even a chemist gets out of practice in weighing at the ordinary analytical balance; a modern microbalance requires a ritual that has to be painfully acquired. No one can expect to take a new method into the laboratory and apply it without going through a long series of control tests on known amounts of the material under study, together with blank tests to discover if his particular set of reagents is above reproach. Furthermore there is no justification for dismissing a new method that has failed to work until one is certain that he has followed directions implicity and exactly. The converse of these propositions is equally important. No chemist should publish a method until he has made certain, by many tests, that it will do what he claims for it, and his obligation for concise and accurate description is, of course, supreme.

I wish to turn now for a moment to the other important function that I think the chemist can fulfil in his connection with plant physiology, namely, that of criticism. It is always interesting to read of the advance that someone has made in unraveling the chemical reactions that take place in the living cell. But there is also the obligation to examine the method by which the results were obtained to see if it really is capable of yielding an unequivocal answer. May I cite an example? A few years ago it was noted in our laboratory,[4] that the quantity of ammonia yielded by mild acid hydrolysis of extracts of tobacco leaf varied widely with the concentration of the acid, if hydrochloric acid were employed. The procedure was designed to determine the quantity of amide nitrogen in the extracts; the answer pro-

[4] VICKERY, H. B., and PUCHER, G. W. Jour. Biol. Chem. 90: 179–188. 1931.

vided by the experiment was that the quantity varied according to the strength of the reagent—a manifestly absurd result. The difficulty was soon traced to the nitrate which is present in unusually high concentration in tobacco leaf extracts. This reacted with the hydrochloric acid and some easily oxidized constituent of the extract to yield ammonia. The difficulty disappeared when we employed sulphuric acid. As a result of this experience I always note carefully the reagent that is employed for the hydrolysis of amides when I see a paper on this subject. If the author used hydrochloric acid, and failed to demonstrate the absence of nitrate, I am compelled to discount the accuracy of his determinations and consequently of his conclusions as well.

A rather striking example of the necessity for critical examination of published results occurs in the splendid review of organic acid metabolism recently published by BENNET-CLARK.[5] The author quotes a great deal of experimental work on the change in the acid content of leaf tissues when these are placed under various conditions. He discusses the changes as if they were due to variations in the proportion of malic acid, although what was measured was the titratable acidity. He may be correct in his assumption, but there is no demonstration of this. It is certain that the titratable acidity changed, but there is no certainty that the malic acid was responsible—this is pure assumption, consequently the theoretical interpretation loses much of its force. BENNET-CLARK himself draws attention to the necessity for critical examination of experimental conditions. He points out that many of RAISTRICK'S supposedly aerobic mold cultures were really grown for part of the time under anaerobic conditions inasmuch as air was circulated over the solution in the plugged flasks only once a day.

Considerations such as these impel me to believe that the plant physiologist can ill afford to dispense with the coöperation of the chemist. In fact I am prepared to go further. If plant physiology proposes to advance beyond the stage of a purely descriptive science, if it is really the aim of this science to account as far as possible for the phenomena of life, and growth, and reproduction in the plant world without resort to vitalistic hypotheses, there is no question in my mind but that the attack on the problem must be made by chemical methods.

The major obligation in life of many, perhaps most, of my audience is that of teaching. You have in your hands the preparation of the younger workers for the investigations that lie ahead. Allow me to urge you to place the greatest emphasis of which you are capable upon the necessity for thorough preparation in chemistry. I should subordinate this only to the training in that most difficult of all human activities, clear and logical thinking.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

[5] BENNET-CLARK, T. A. New Phytol. 32: 32–71, 128–161, 197–230. 1933.

A LARGE-CAPACITY DRYING OVEN WITH CONSTANT UNIFORM TEMPERATURE AND FORCED VENTILATION

EMMETT V. MARTIN

(WITH ONE FIGURE)

Temperature and rate of drying are important factors in desiccation of plant material for analysis. Various temperatures, ranging from 30° to 105° C., have been used for this purpose, although there is no particular one which is entirely satisfactory in all respects. In the higher range there may be a decrease of soluble nitrogen by coagulation, and caramelization of the sugars may occur, while at the lower values there will be enzyme action and respiration. TOTTINGHAM and LINK[1] have made a special study of methods of desiccation of plant tissue, and have given data showing that preparation under widely different conditions yields results that are not comparable. They conclude that material which dries readily undergoes least destructive change with a temperature of about 65° C. When the material is dried in an oven with very little ventilation, the humidity soon becomes so high that drying proceeds very slowly, thus allowing destructive action to continue for a longer period of time. If one wishes to compare analyses of stems and leaves, the former should be sliced longitudinally into slender strips so that they will dry more quickly. Leaves also can be cut into a number of pieces to similar advantage. When the material is placed in the oven, it should be well spread out to allow good circulation of air around all parts.

In the preparation of plant tissue for analysis, therefore, it is desirable to use an oven that has a fairly constant uniform temperature and good ventilation. Furthermore, if considerable quantities are to be dried, a rather large capacity is needed. Such ovens are obtainable, but the expense may in some cases be prohibitive. It is the purpose of this paper to describe an oven that can be constructed by local sheet-metal workmen at much lower cost than its equivalent can be purchased from manufacturing companies.

In figure 1 is shown a diagram of a cross-section through the center of the oven, drawn to scale. It is made up of three separate sections, *A*, *B*, and *C*. Section *A* comprises the oven proper, *C* contains the heaters, and *B* provides extra insulation between *A* and *C*. The outside dimensions of

[1] LINK, K. P., and TOTTINGHAM, W. E. Effects of the method of desiccation on the carbohydrates of plant tissue. Jour. Amer. Chem. Soc. 45: 439–447. 1923 (also 47: 470–476. 1925).

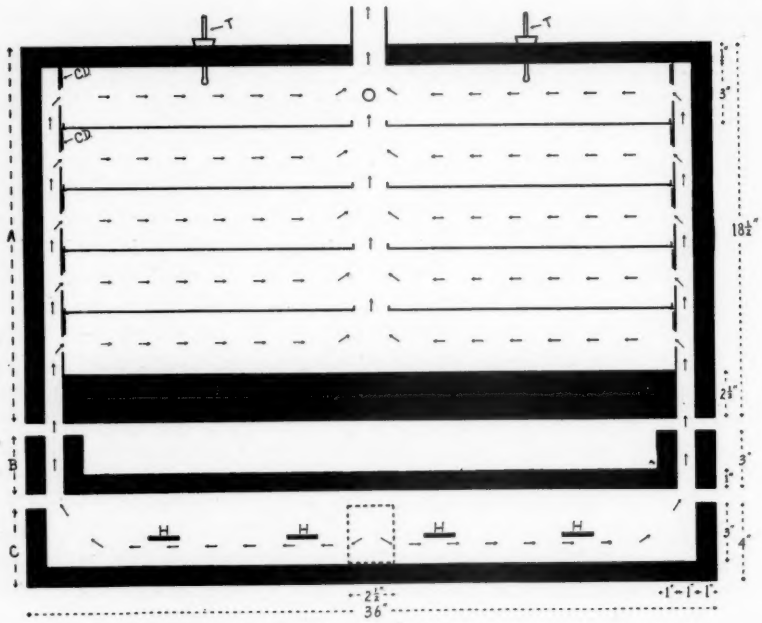

FIG. 1. Diagram of cross-section through center of oven, drawn to scale.

section A are $36'' \times 20'' \times 18\frac{1}{2}''$, the inside are $32'' \times 18'' \times 15''$, while others are shown in the illustration.

The walls are constructed of no. 26 galvanized sheet-iron of two layers 1″ apart, except on the floor of section A where the spacing is $2\frac{1}{2}''$. The spaces between layers are filled at the rate of 12 pounds per cubic foot with loose rock wool, manufactured by the Gimco Rock Wool Products Co. The four shelves in section A are made of no. 24 galvanized sheet-iron, with the edges bent over $\frac{1}{4}''$ for added strength. In the center of each shelf and in the top of the oven there is a slot $1\frac{1}{2}'' \times 16''$ to allow for the passage of air. The shelves rest on slides at each end of the oven and are removable.

The amount of air that passes over each shelf is controlled by a diaphragm opening at each end (fig. 1). These openings are $\frac{3}{4}'' \times 16''$ and can be partially or completely closed by means of covers (C, D). These covers are adjustable and are held in place by means of a thumbscrew in a slot at each end.

Heat is furnished by four coils (H) of no. 26 B. & S. gauge chromel A wire wound on strips of transite $\frac{1}{4}'' \times 1\frac{1}{2}'' \times 18''$, supported in place by angle irons. The two outer coils are connected in series and draw 405 watts from a 110-volt A.C. source, while the two inner ones, also in series, take 235

watts. When the oven is in operation the 405-watt coils are heating continuously, but the 235-watt coils are controlled by a DeKhotinsky bimetallic thermoregulator which projects through the rear wall into the center of the space above the top shelf (shown as small circle in figure 1). Electrical connections to the heaters are made through binding posts insulated from the walls by fiber tubing and washers.

Ventilation is produced by a no. 1 baby conoidal blower manufactured by the Buffalo Forge Co. This blower is mounted on a small platform attached to the rear of section *C*. Air is passed from the blower to this section through a short metal tube, which is round at the blower end and square at the oven end. In this tube is mounted an adjustable damper for the control of air flow. The blower has a capacity of 78 cubic feet of air per minute, but its delivery is limited in this case to approximately 12 cubic feet per minute. With this rate of flow the air in the oven is replaced more than twice per minute.

The course of air through the oven is indicated in the figure by small arrows. Leakage between the sections is prevented by padding the points of contact with asbestos, and the sections are prevented from slipping on each other by flanges. The diaphragm covers (*C, D*) are adjusted so that approximately the same amount of air passes over each shelf. To accomplish this, the top covers are nearly closed and the bottom ones wide open; the openings of the others are graduated accordingly.

Between sections *A* and *B* is an air space open at the front and back to the outside. This space and the thick floor of *A* effectively prevent any transfer of heat from the heaters through the floors to the lower shelf. This is necessary in order to obtain uniform temperature in the oven.

Measurements of the temperature distribution within the oven were made by means of six thermocouples operating simultaneously at different points. Readings were taken every few minutes over a complete cycle of opening and closing of the thermostat contacts. The couples were then moved to other points and further readings taken. This process was repeated until all regions of the oven had been covered. The maximum difference in temperature found was 7° C., and its variation at any particular point was not more than 2° C. from the mean at that point. During these tests the thermostat was set at approximately 65° C., registered by thermometers marked *T* in figure 1.

Summary

1. For preparation of plant tissues for analysis, it is desirable to have a drying oven of fairly constant uniform temperature, good ventilation and large capacity.

2. The oven described has the following advantages and characteristics:

a) It can be made by local sheet-metal workmen at much lower cost than its equivalent can be purchased from manufacturing companies.

b) It has a volume of 5 cubic feet and a shelf space of 20 square feet.

c) No two points differ in temperature by more than 7° C.

d) The temperature at any particular point does not vary more than 2° C. from the mean at that point.

e) The air is replaced more than twice per minute.

f) Its maximum power requirement is 640 watts.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

BRIEF PAPERS

QUANTITATIVE ABSORPTION SPECTRA OF THE COMMON CAROTENOIDS[1]

(WITH ONE FIGURE)

The specific absorption coefficients in the visible spectrum have been determined for alpha carotene, beta carotene, leaf xanthophyll, and lycopene[2] in solution. These carotenoids were prepared by the best available

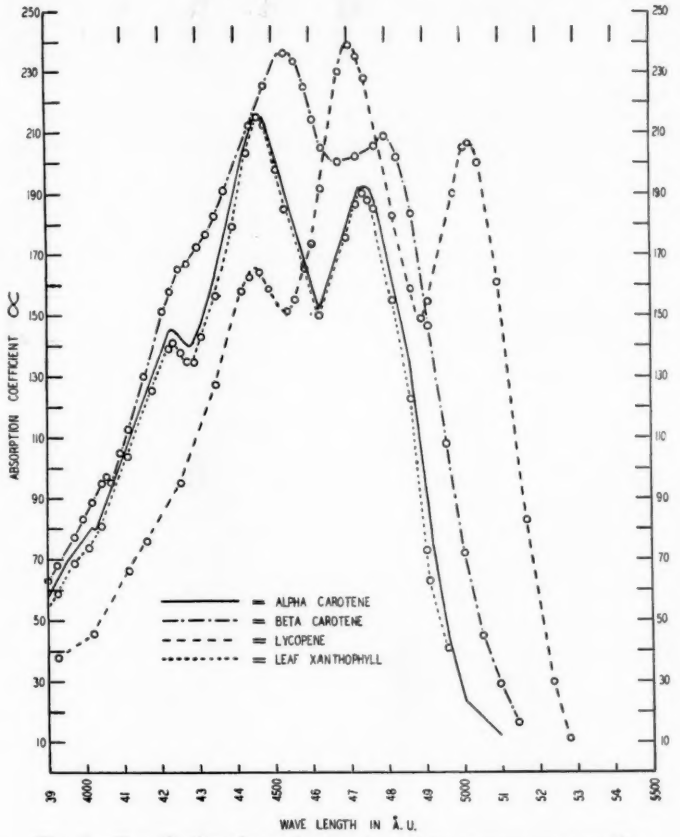

FIG. 1. Quantitative absorption spectra of the common carotenoids.

[1] Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

[2] Lycopene sample was purified and furnished by M. B. MATLACK, Bur. of Chem. and Soils. United States Department of Agriculture.

chromatographic methods, care having been taken to exclude oxygen in the important purification processes. The specific absorption coefficients (fig. 1)[3] were later used as the standards of purity of the various samples employed. This method being more sensitive and therefore more reliable than either of the methods employing the melting-point or optical rotation—although the latter two were used in the earlier stages of purification.

The solvent employed was 80 per cent. absolute ethanol and 20 per cent. anhydrous diethyl ether. All of these carotenoids were sufficiently soluble in this solvent. The specific absorption coefficients were determined with an accuracy of less than 1.3 per cent. error, by a method (Jour. Phys. Chem. **38**: 1–11. 1934.) which employs the photoelectric cell to determine the light intensities.

By selecting suitable wave-lengths (fig. 1), accurate quantitative spectral analyses of ternary mixtures of alpha and beta carotene or leaf xanthophyll, beta carotene, and lycopene have been made with an error of less than 2.5 per cent. It is expected that ultimately a procedure will be developed whereby all the photosynthetic pigments may be analyzed in the presence of each other by this optical method.—ELMER S. MILLER,[4] *University of Chicago.*

[3] In Plant Physiol. 9: 179. 1934: It is obvious that the carotene with the maxima farthest toward the infra-red should be beta carotene instead of alpha carotene.

[4] National Research Fellow in the Biological Sciences.

NOTES

Annual Election.—The secretary-treasurer, Dr. A. E. MURNEEK, has announced that the Society has chosen Dr. BURTON E. LIVINGSTON, Johns Hopkins University, as its president for 1934–1935. The vice-president elect is Dr. JAMES P. BENNETT, University of California. This happy outcome of the election insures an auspicious inauguration of the Society's second decade of service to botanical science. All members will wish to enter into active cooperation with the officers to plan and to achieve ever more important objectives.

Pittsburgh Meeting.—Plans for the Pittsburgh meeting are being formulated by the program committee. Every member who can do so should make arrangements to attend this meeting. It is the eleventh annual meeting of the Society, and is centrally located for the eastern and middle western members. The meetings have become more and more valuable each year, so that one can hardly afford to miss them. When the officers call upon members for service in connection with the meetings, it is hoped that no one will refuse to share in the responsibilities, privileges, and duties of membership. As full information as possible will be carried in the October number of PLANT PHYSIOLOGY.

Berkeley Meeting.—The summer meeting held in connection with the meeting of the A. A. A. S. at the University of California during the week of June 19–23, was well attended, and much enjoyed by those whose good fortune it was to be present. It brought together most of the plant physiologists of the western states, with a modest attendance from the east. The papers submitted were pooled with those of a physiological nature submitted to the Botanical Society of America and three joint half-day sessions were held. A wide variety of subjects were discussed in the thirty-four papers presented. Of especial interest was a symposium on absorption conducted by D. R. HOAGLAND, S. C. BROOKS, L. R. BLINKS, F. C. STEWARD, and PIERRE PREVOT. Several papers on absorption were read also in the regular sessions so that this subject proved to be the outstanding one at this meeting. Attendance at the physiological sessions was very good, as many as one hundred being present at times. Two excellent and interesting public addresses of interest to physiologists were delivered on Thursday afternoon by Prof. GÖTE TURESSON of the University of Lund, Sweden, and Prof. H. C. THOMPSON of Cornell University.

An interesting feature of the meetings was a series of open-house exhibits and demonstrations by various departments of the University. Of

especial interest to plant physiologists were those showing the greenhouse and laboratory equipment and the experimental work under way by the Department of Botany and the College of Agriculture. The week ended with a number of excursions to such points of interest as Stanford University, University Farm and Agricultural Laboratories at Davis, California Academy of Sciences and Golden Gate Park, Muir Woods, Yosemite Park, and Mariposa Grove.

New England Section.—The first meeting of the New England Section at Amherst on May 25–26, 1934, was decidedly successful. The attendance was close to 75, which compares favorably with the number in attendance at annual meetings of the Society as a whole. The programs consisted of sixteen papers, eight on Friday afternoon, and eight on Saturday morning. A dinner was held at Draper Hall on the campus of the Massachusetts State College, Friday evening. Dr. C. G. DEUBER, Yale University, presided at the banquet, and the two speakers were Dr. H. P. BAKER, President of Massachusetts State College, and Dr. F. A. WAUGH, Professor of Landscape Architecture. It was a very happy occasion for every one, and the meeting throughout was marked with optimistic enthusiasm. The 1935 meeting is to be held at the University of New Hampshire, Durham, New Hampshire. This Section is enjoying a well-deserved success, and setting an example that members in other regions may wish to emulate.

Chemical Methods.—Only about 35 sets of the reports of the Chemical Methods Committee remain unsold. There may be among the more recently elected members some who would appreciate owning a set of these reprints with their valuable summaries of analytical technique and their literature lists. As long as they last they can be obtained from Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building, University of Wisconsin, Madison, Wisconsin. Those who want them should act quickly, before the supply is completely exhausted.

Directory.—The secretary-treasurer expects to issue a new membership directory during the coming year. Data will be required from each member in order to make it quite accurate and up-to-date. When information is requested for this directory, it is imperative that it should be furnished promptly. Dr. MURNEEK asks your hearty cooperation in getting together the material necessary for the membership list.

Endowments.—Two of the three endowment funds of the American Society of Plant Physiologists continue to increase slowly. The CHARLES REID BARNES life membership endowment fund contains nearly $1,475, and

the general endowment is now $175. The STEPHEN HALES prize award endowment fund remains at $1,100. Gifts to these endowments may be made at any time. Even small gifts are handled in such manner that they begin to yield interest toward the objectives of the endowments immediately upon receipt by the secretary-treasurer. Other scientific organizations have found that permanent endowments are necessary to their permanence and sound development. The financial arrangements of the Society contemplate a permanently growing endowment, and it is a privilege to contribute to the financial stability of its work. This privilege any member may share if he so desires.

Charles Reid Barnes Botanical Laboratory.—The botanical laboratory erected at the University of Chicago several years ago has been named the CHARLES REID BARNES Botanical Laboratory by recent action of the Board of Trustees of the University. Dr. BARNES was the first professor of plant physiology at Chicago, and was a leader in the development of experimental laboratory courses in this field. His ability as critic and lecturer, his unflinching courage and inspiring personality endeared him to all of his students and colleagues. He lost his life in February, 1910, at the age of 51 years, as the result of a fall on icy pavements as he attempted to meet an engagement with a graduate group in plant physiology. It therefore seems very appropriate that this laboratory, devoted mainly to plant physiology and plant pathology, should be named for one so intimately associated with the beginning of laboratory training in plant physiology in the United States.

Chlorophyll and Carotenoids for Research.—It is now possible to obtain chlorophyll and carotenoids for research and commercial purposes in almost any quantity desired. The American Chlorophyll Co., 3240–3244 K St. N. W., Washington, D. C., has developed extraction on a large scale, and offers chlorophyll 5 ×, which is claimed to be a highly purified preparation of chlorophylls α and β. The grade 3 × is not so pure, but a good grade. They have also prepared and can furnish salts as follows: sodium magnesium chlorophyllin, sodium iron chlorophyllin, sodium copper chlorophyllin, copper pheophytin, and iron pheophytin. The characteristic alcohol, phytol, is offered in pure and crude forms. Both carotene and xanthophyll may be had in crystalline form, and carotene in oil. One thing that should be done at once is to subject the chlorophyll preparations and the carotenoids to examination by the methods of ZSCHEILE, MILLER, and their co-workers to determine how constant are the proportions of α and β in different samples, and what these newer methods reveal as to actual purity of the preparations. The development of such sources

of material is a highly commendable project. Inquiries may be addressed to Robert H. Van Sant, President of the American Chlorophyll Co.

Plant Biochemistry.—In the July, 1933, number of PLANT PHYSIOLOGY a preliminary notice of a manual of plant biochemistry was published. The completed volume, Plant Biochemistry, By Dr. W. E. TOTTINGHAM, University of Wisconsin, has just been received from the publishers, The Burgess Publishing Co., Minneapolis, Minnesota. It is mimeographed, handsomely bound in green flexible leatherette binding, and contains 219 pages. There are 11 chapters with the following titles: General aspects; the materials of metabolism; the photosynthesis of carbohydrates; the metabolism of carbohydrates; fat metabolism; the metabolism of nitrogen compounds; physicochemical relations of the plant cell; the nature and function of enzymes; chemical aspects of respiration; salt nutrition; and climatic effects in metabolism. The work is a noteworthy attempt at orderly consideration of the chemical processes of plant life. Copies may be purchased at $4.25 each. Orders should be sent directly to the Burgess Publishing Co., 426 So. Sixth St., Minneapolis, Minnesota.

Electrokinetic Phenomena.—A very useful monograph, Electrokinetic Phenomena and their Application to Biology and Medicine, by HAROLD A. ABRAMSON, has been published by the Chemical Catalog Co., New York. The earlier chapters discuss the historical development of electrokinetics, early theories, methods of experimentation, and the more recent theories. Chapter V deals with proteins and related compounds; chapter VI with the general effects of salts on inert surfaces; chapter VII with inorganic surfaces; and chapter VIII with organic surfaces. The last three chapters consider the electrokinetics of gases, the electrical phenomena of blood cells, spermatozoa, tissues, etc.; and bacteria, antibodies, and viruses and related systems. There are several appendices, followed by author index and subject index. It is an American Chemical Society Monograph, no. 66, bound in the same style as other monographs of this series. Much of the information will be useful to plant physiologists, although the applications are most frequently made in the zoological and medical fields. The problems dealt with are of universal occurrence, and concern the materials with which any of us work. The price of the book is $7.50 per copy.